# EFFECTS OF MICROBIAL ECOLOGY AND INTESTINAL MORPHOLOGY ON ENERGY UTILIZATION IN ADULT COCKERELS

A Dissertation

by

OMAR GUTIERREZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2009

Major Subject: Poultry Science

# EFFECTS OF MICROBIAL ECOLOGY AND INTESTINAL MORPHOLOGY ON ENERGY UTILIZATION IN ADULT COCKERELS

A Dissertation

by

OMAR GUTIERREZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

| | |
|---|---|
| Chair of Committee, | Christopher A. Bailey |
| Committee Members, | A. Lee Cartwright |
| | John B. Carey |
| | David J. Caldwell |
| | Delbert M. Gatlin III |
| Head of Department, | John B. Carey |

August 2009

Major Subject: Poultry Science

# ABSTRACT

Effects of Microbial Ecology and Intestinal Morphology on

Energy Utilization in Adult Cockerels.

(August 2009)

Omar Gutierrez, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Christopher A. Bailey

A series of four experiments were conducted to evaluate whether a preconditioning period, in which adult leghorn cockerels are allowed 3 weeks to adapt to diets containing relatively high levels of guar meal (GM) resulted in changes in digestive tract morphology and ecology. It was expected that these changes would result in increased utilization of GM as birds became acclimated to consuming it at high levels. In the 1st experiment, 28 birds were fed diets containing 0, 6, 12, or 24% GM. Consuming the 24% GM diet resulted in decreased body weight, increased small intestine, liver, and pancreas weight, increased ceca and villus length, and increased severity of injury to the intestinal mucosa ($P < 0.05$). In the 2nd experiment, 28 cockerels were subjected to a partial cross-over experiment. Preconditioning to the 24% GM diet resulted in decreased TMEn of GM, decreased body weight, and decreased absorptive capacity of the GI tract as evidenced by increased energy lost via the excreta ($P < 0.05$). The 3rd experiment evaluated how differences in endogenous energy losses (EEL) from 30 cockerels affected the TMEn content of GM in birds consuming 0, 6, or 12% GM. Decreased EEL

was observed in birds conditioned to 6 and 12% GM relative to the control group ($P < 0.05$). Further, decreased TMEn of GM was observed in birds consuming 6% GM relative to those consuming the control diet ($P < 0.05$). In the 4$^{th}$ experiment, changes in cecal microbiota were evaluated in 24 cockerels consuming 0, 6, or 12% dietary GM using denaturing gradient gel electrophoresis of amplified bacterial DNA sequences. Cecal microbiota of birds consuming 12% GM was considerably different from the control group (similarity coefficient = 84%) with an apparent decrease in the complexity of microbial communities. Results of these studies show that consuming 12% dietary GM results in changes in the cecal microbial community which may be responsible for modest increases in the TMEn of GM when compared to 6% dietary GM, which consistently resulted in decreased TMEn values of GM. Further, determining the TMEn value of GM appears to be highly dependent upon factors such as preconditioning diet and timeframe and innate physiological “thresholds” which seem to initiate or limit the maximum physiological response to preconditioning that the bird is capable of.

# DEDICATION

For my father,

you are dearly missed…

# ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to my committee chair, Dr. Christopher A. Bailey for the encouragement, wisdom, and direction offered during my stay at Texas A&M, and to my committee members, Dr. A. Lee Cartwright, Dr. John B. Carey, Dr. David J. Caldwell, and Dr. Delbert M. Gatlin III, for their advice and guidance throughout the course of this research.

Special thanks also go to Dr. Akram Ul-Haq, Dr. C.R. Creger and Mr. Dale Hyatt for their unwaning support in helping to achieve my academic and personal pursuits, and to Dr. Michael Hume, Dr. Phelue N. Anderson, and Mr. Charles Hernandez for their technical training and assistance. I also want to extend my gratitude to the Hispanic Leadership Program in Agricultural and Natural Resource Sciences Fellowship and Tom Slick Graduate Research Fellowship, both of which greatly assisted with funding of my educational endeavors.

# TABLE OF CONTENTS

CHAPTER Page

# LIST OF FIGURES

# LIST OF TABLES

# CHAPTER I

# INTRODUCTION

Annually, over two billion tons of cereal grains and 150 million tons of oil seeds are produced globally, which yield approximately 250 million tons of fibrous by-product materials. These materials are typically the by-product of lipid extraction from oilseeds (e.g. soybean, canola, and sunflower meals) or carbohydrate extraction from grains (e.g. dried distillers grains, corn gluten and guar meal). The fiber component of these ingredients is primarily composed of non-starch polysaccharides (NSP) which form part of the cell wall structure in most cereals, and serve as an energy store for embryo germination and development in legumes. Recently, the role of fiber in monogastric animal diets has attracted increased attention due to the fact that NSP are poorly utilized and in many cases elicit an anti-nutritive effect. These factors are of great concern because the world's human population continues to increase whereas its grain production levels remain relatively static, placing increased pressure on the need for improved utilization of nutrients derived from plant sources. Therefore, more efficient utilization of ingredients which historically have been considered low quality is of increasing concern to the long-term sustainability of agriculture.

Digestibility can be defined as the ability of a food to be digested into a form in which it can be absorbed from the gastrointestinal (GI) tract for use in the body of an animal. Accordingly, the digestibility of an ingredient determines its value as a

---

This dissertation follows the format of Poultry Science.

feedstuff. Highly digestible ingredients provide greater nutrient availability, and thus command a higher price than poorly digestible ingredients. Due to the lack of endogenous enzymes capable of degrading complex carbohydrates, many monogastric animal feeding programs utilize large volumes of highly digestible feed ingredients as well as exogenous dietary enzymes in an effort to optimize animal production parameters, but these approaches ultimately add to the cost of a diet. Inclusion of less expensive, usually less digestible ingredients is enticing from an economic perspective, so long as negative effects associated with lesser quality ingredients are remediated.

Most monogastric animal diets utilize legume by-products as the primary plant protein source due to their high rates of digestibility and generally well-balanced amino acid profiles. However, in addition to protein most legume products also contain considerable amounts of NSP which are composed primarily of mannans, galactans, and pectic polysaccharides. Guar (*Cyamopsis tetragonoloba*) is a drought tolerant legume that is widely cultivated in India and Pakistan. It is grown for the highly viscous gum contained within the endosperm of its seeds. Guar gum (GG) is used as a thickening agent in the food, paper, cosmetics, and petroleum industries. Guar gum is a heavily branched galactomannan oligosaccharide that is comprised of β-1→4 mannose backbone with α -1→6 galactose side chains (Van Nevel et al., 2005). Guar meal (GM) is comprised of a blend of high-protein endosperm and a low-protein hull fraction that typically contains approximately 35% crude protein and 18% residual GG (Bakshi et al., 1965). Guar meal market value highly correlates with the soybean meal market and generally sells for about 60% of that of dehulled soybean meal.

Guar meal's use as an ingredient in poultry diets has been limited because high dietary inclusion rates depress feed consumption and growth rates, decrease body weight, and increase mortality in broilers (Borchers and Ackerson, 1950; Sathe and Bose, 1962; Bakshi et al., 1964; Couch et al., 1967; Saxena and Pradhan, 1974; Patel and McGinnis, 1985). When added into the diet, GG increases intraluminal viscosity and decreases gastrointestinal passage rates. Additionally, GG benefits GI physiology through prebiotic functions, hypocholesteremic properties, and immunostimulatory qualities (Favier et al., 1997; Dario-Frias and Sgarbieri, 1998; Favier et al., 1998; Moriceau et al., 2000; Yamamoto et al., 2000). Furthermore, GG affects the intestinal microenvironment by limiting adhesion of pathogenic bacteria to the intestinal epithelium of the host and, by creating a more anaerobic environment within the intestine which favors proliferation of "beneficial" bacterial species.

Studies involving wild galliform species indicate that a significant portion of the daily energy requirement is derived from microbial fermentation of plant fibers. Ruffed grouse, Chukkar partridge, and Bobwhite quail respond to high cellulose diets by physiological modification of the digestive organs (i.e., lengthening of the ceca and intestinal tract, and enlargement of the gizzard, liver and pancreas) (Inman, 1973) which has been associated with consuming a less nutrient-dense diet. Studies evaluating seasonal changes in the digestive system of many galliform species report striking differences in nutrient utilization rates depending on time allowed for diet acclimatization (Fenna and Boag, 1974). Additionally, changes in microbial population in response to fiber have been reported in several monogastric species (Fernandez et al.,

2002). However, these changes require a period of time in which the animal adapts to the lower-quality, high fiber diet.

Historically, digestibility studies have been conducted using animals which have not been acclimatized to poorly digestible diets prior to experimentation (i.e., animals which have been preconditioned to low-residue, highly digestible diets). In these studies, physiological responses of the GI tract as well as the development of an intestinal microbiota more capable of fiber digestion has been limited, thereby penalizing fibrous feed ingredients relative to those which are more easily digested. This results in data which may not be accurate with respect to animals given appropriate diet acclimation periods.

In a preliminary study conducted in our laboratory, the true metabolizable energy (TME) of GM was determined according to the diet substitution method proposed by Sibbald and Slinger (1962) with one notable exception. The birds in this study were subjected to increasing levels of GM over time, effectively conditioning them to a diet high in soluble fiber over a period of 3-4 weeks whereas, Sibbald and Slinger used a different group of birds for each respective level of substitution. Our results indicated an increase in energy utilization of GM occurring approximately 3 weeks into the trial period when the birds were consuming a diet consisting of between 10 and 30% GM (Figure 1-1) which is similar to observations in wild galliforms consuming diets high in fiber. This observation formed the basis of my research hypothesis which was that

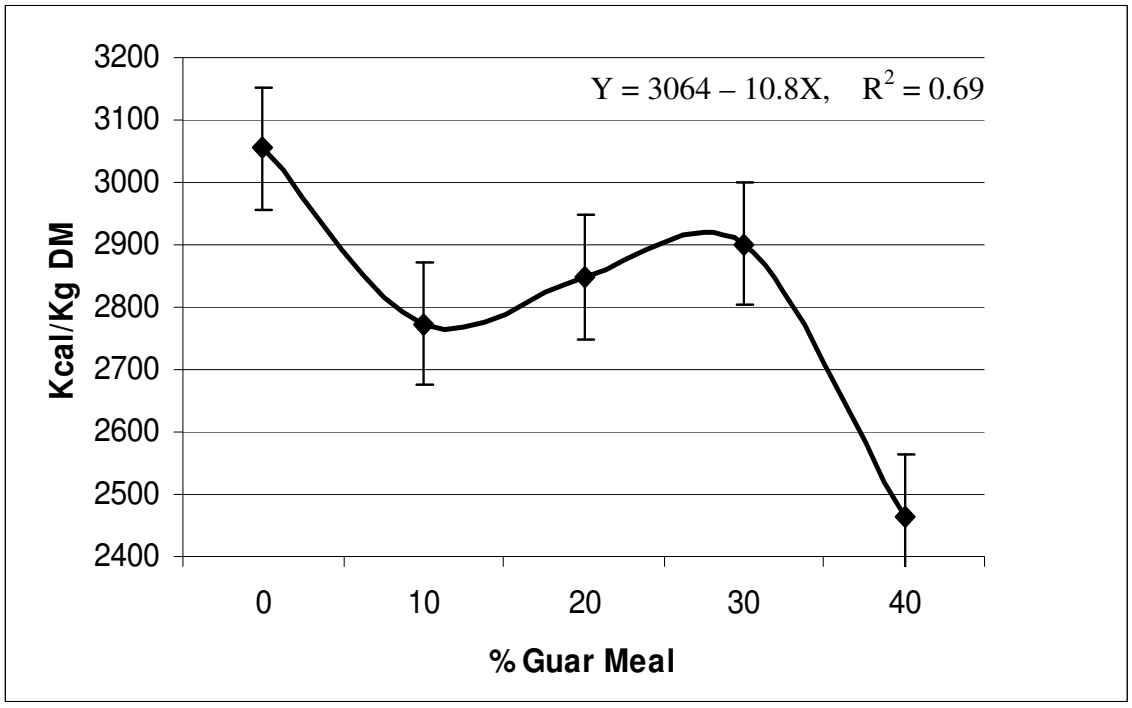


**FIGURE 1-1. True metabolizable energy (TME) of guar meal (GM) fed to the same group of cockerels at increasing dietary levels.**

chickens pre-conditioned to consuming large amounts of GM would increase nutrient digestibility over time. The objectives of this research were: 1) to determine the level at which diets containing relatively high levels of guar meal elicit a change in digestive system anatomy and intestinal morphology in chickens; 2) to determine whether a diet pre-conditioning period of 3 weeks results in significant changes in the True Metabolizable Energy and nitrogen digestibility of GM; and 3) to evaluate differences in the microbial ecology of the ceca of chickens consuming differing levels of GM over a 3-week period of time.

# CHAPTER II

# LITERATURE REVIEW

## NON-STARCH POLYSACCHARIDES IN MONOGASTRIC DIETS

### Classification and Chemistry

Polysaccharides are polymers of monosaccharides joined through glycosidic linkages and are classified in terms of their structural components, including the identity of the monosaccharides present in the structure, the monosaccharide ring forms, and the positions and configurations of glycosidic linkages present in the compound. The primary energy-yielding non-lipid component in commercial poultry diets is starch. The predominant monosaccharides in poultry diets are glucose and fructose, while the predominant disaccharide is sucrose (Larbier and Leclercq, 1992). There is usually no difficulty digesting starch in the GI tract. However, digestion of non-starch polysaccharide (NSP) fractions tends to be more troublesome due to a lack of endogenous digestive enzymes capable of their degradation (Choct and Annison, 1992).

The term NSP covers a large variety of polysaccharide molecules excluding α-glucan (starch) structures. This classification is based on the original methodology for extracting and isolating polysaccharides in which the remaining residue after a series of acid and alkaline extractions of plant material was called cellulose, while the fraction of this residue solubilized by alkali was called hemicellulose. More recently, classification by solubility in either neutral- or acid-detergent solutions was proposed by Van Soest et al. (1991). While this system has proven useful for the purposes of ruminant nutrition, it

lacks precision with respect to chemical structure as well as biological function in monogastric animal diets.

The NSP found in cereal grains and their by-products is composed predominantly of xylans, β-glucans and cellulose. Cereals such as corn and sorghum contain very low levels of NSP relative to wheat, rye, and triticale which contain substantial amounts of both soluble and insoluble NSP. Unlike cereal grains, the NSP content of legumes is comprised primarily of pectic polysaccharides and mannan or galactan oligosaccharides rather than cellulose and xylans. The NSP content of a given feed ingredient varies not only between different ingredients but also between the same ingredient due to variety and geographic location. However, the chemical structure of NSP will not change in a particular ingredient due to these factors. Generally, NSP can be divided into three major categories, including cellulose, non-cellulosic polymers, and pectic polysaccharides (Bailey, 1973).

Although the effect of NSP in monogastric diets can be quite diverse depending upon the specific compound that is administered, it is generally accepted that the major detrimental effects are associated with the viscous nature of these polysaccharides, their physiological and morphological effects on the digestive tract, and their interaction with the microbiota of the gut (Choct, 1997). The mechanisms include altered intestinal transit time, modification of the intestinal mucosa, and changes in hormone regulation due to variable rates of nutrient absorption (Vahouny, 1982).

**Viscosity**. The main problems associated with feeding NSP to poultry are viscosity and water-holding capacity. Research shows that changes in viscosity are due

to soluble pectins or gums that can greatly increase intestinal viscosity even at very low levels of inclusion (Annison and Choct, 1991). Insoluble polysaccharides such as cellulose and lignin can hold water, but their use has little effect on viscosity (Smith and Annison, 1996). Addition of certain NSP fractions to poultry diets reduces digestion of starch, protein, and lipids. It has been suggested that this is primarily associated with the impairment of diffusion and transport of dietary enzymes within GI contents and across the intestinal epithelium (Smith and Annison, 1996).

The viscosity of a polysaccharide depends on its solubility and molecular weight. In turn, solubility depends on the chemical structure of the polysaccharide and its association with the rest of the components of the plant cell. The presence of NSP in the GI tract prolongs gastric emptying time and retards absorption of nutrients. Diets that contain a substantial amount of isolated, complex carbohydrates tend to be bulky and require longer times for digestion, slowing GI passage rates more so than a mixed, complete diet, suggesting a "symbiotic-type" relationship between various fiber sources, lipids and proteins (Eastwood et al., 1986).

Rates of nutrient release from NSP are influenced by factors such as the affects of ingredient processing and the intactness of epithelial tissues. While no evidence suggests that viscous polysaccharides inhibit transport across the intestinal epithelium, their properties inhibit access of nutrients to the epithelial tissue layer. Two mechanisms bring nutrients into contact with the epithelium: 1) intestinal contractions create turbulence that mix the luminal contents and bring material from the center of the lumen close to the epithelium; and 2) nutrients then diffuse across the thin unstirred layer of

fluid lying adjacent to the epithelial wall (Johnson and Gee, 1981). Increasing the viscosity of luminal contents impair both luminal turbulence and nutrient diffusion across the unstirred layer (Edwards et al., 1988). It has been suggested that β-glucans found in barley and oats create complex bonds with digestive enzymes and decrease their activities (Ikeda and Kusano, 1983). However, Ikegami et al. (1990) showed that the activity of GI tract enzymes in rats increased after feeding diets containing viscous ingredients. The fact that the viscous property of NSP is a major factor in the anti-nutritive effect of NSP in monogastric animals is supported by the wide-spread use of exogenous enzymes in their diets. These enzymes cleave otherwise indigestible NSP molecules into smaller polymers, thereby reducing the viscosity of GI tract contents and increasing digestibility of feed (Bedford et al., 1991).

**Interaction with GI Microbiota**

Non-digestible oligosaccharides which support the growth of certain cecal or colonic bacterial genera can be classified as "prebiotic." These polysaccharides exert beneficial physiological functions by two mechanisms, by promoting growth and proliferation of beneficial bacteria in the GI tract, and by preventing adhesion of pathogens to epithelium of the GI tract. Beneficial or "probiotic" bacteria are strictly anaerobic, lactic acid-producing genera which include *Lactobacillus spp*. and *Bifidobacterium spp.* among others. The presence of these bacterial species in the GI tract results in the production of large amounts of short chain fatty acids (SCFA), which decrease luminal pH and exert an inhibitory affect on growth and proliferation of pathogenic microorganisms (Noack et al., 1998). In addition to producing SCFA,

probiotic bacteria improve the health of the host by enhancing digestive enzyme activities, competing against pathogenic bacteria for nutrients and epithelial adhesion sites, and stimulating immune functions of the host (Fuller, 1989).

Although they are poorly digested by monogastric animals, dietary NSP can affect the intestinal ecosystem by providing a fermentable substrate which promotes growth of beneficial bacterial species within the GI tract, and by preventing adhesion and subsequent colonization of pathogenic bacteria to the epithelial tissues of the GI tract. Undigested or partially-digested NSP that reaches the distal GI tract represents a nutrient source that intestinal bacteria can use to synthesize short-chain fatty acids (SCFA) which are of metabolic importance to the host animal. In poultry, short-chain fatty acids are readily absorbed by the colonic mucosa, though only acetic acid reaches the systemic circulation in appreciable amounts (Kirgchgessner et al., 1999). In humans, butyric acid supplies up to 70% of the energy needed for growth and differentiation of the colonic epithelium (Roediger, 1980). The production of SCFA is also important in maintaining a slightly acidic intestinal environment that suppresses the growth of pathogenic bacteria in the ceca of chickens (Topping, 1996).

The cecal bacterial flora is dependent upon endogenous and dietary nutrient sources. The amount of substances passing through the intestine to the colon varies in an inverse relationship between cecal bacterial metabolism and upper intestinal nutrient transport. Non-starch polysaccharides also have an influence on bacterial mass and enzyme activity with certain types of NSP (e.g. mannan oligosaccharides) preventing adhesion of pathogenic bacteria to the GI tract by not only competing for epithelial

surface area, but also by irreversibly binding to complexes present on the bacterial cell membrane, thereby inhibiting their ability to bind to the intestinal wall.

Under normal conditions, facultative anaerobes dominate the microbiota of the small intestine and strict anaerobes make up the cecal microbiota of the chicken (Salanitro et al, 1978). The viscous nature of NSP tends to increase the residence time of digesta in the intestine, which decreases oxygen concentration and thereby promotes the development of strict anaerobic bacterial species, specifically *Lactobacillus spp*. and *Bifidobacterium spp*. (Noack, et al., 1998).

**Modification of GI Tract Morphology**

Soluble NSP acts not only as a prebiotic substrate for monogastric animals, but also changes GI tract function by modifying endogenous secretion of water, proteins, electrolytes, and lipids. As dietary bulk increases, the digestive system is put under increased pressure to move the more viscous bolus through the alimentary canal as well as to draw energy and nutrients from a less concentrated, bulky diet.

Prolonged consumption of NSP is associated with significant adaptive changes in the digestive system of rats (Ikegami et al., 1990) and many birds (McNab, 1973). These changes are characterized by the enlargement of the digestive organs and increased secretion of digestive fluids, accompanied by an eventual increase in nutrient digestibility. Redig (1989) suggested that feeding NSP to poultry may have a negative impact on production as increased fermentation in the ceca may lead to cecal hypertrophy creating a relative increase in maintenance energy requirement associated with the enlarged organs. Further, the author proposes that this increase in size may be

due to a number of factors including prolonged residence of fiber in the ceca, increased bacterial mass, or increased bacterial metabolic end products. Similarly, Miller (1976) found that mallard ducks fed low- and high-fiber (alfalfa) diets showed an increase in cecal size when consuming pelleted diets.

An important response of many NSP molecules in the diet is to reduce reabsorption of bile acids in the ileum, thereby increasing the amount of bile acids and fats reaching the colon. This property enhances bile acid secretion and results in increased loss of these acids in the feces (Ikegami et al., 1990). This results in increased hepatic synthesis of bile acids from cholesterol in order to maintain the pool of these metabolites in the entero-hepatic circulation thereby reducing serum cholesterol (Yamada et al., 1999). Ultimately, this can lead to decreased digestion and absorption of dietary lipids, which are dependent upon the formation of mixed micelles (Cummings and Macfarlane, 1997).

## GUAR MEAL

### Nutrient Content and Use

Guar, *Cyamopsis tetragonoloba,* is a drought-tolerant, annual legume grown for its high concentration of galactomannan gum which is used as a thickener in the food, cosmetics and pharmaceutical industries. The majority of domestic needs for guar gum are currently met by imports from India and Pakistan, as demand far exceeds U.S. guar bean production. The imported product is usually in the form of guar splits, which is the unprocessed endosperm fraction of the guar bean. The by-product, guar meal, usually

remains in its country of origin and is typically used as animal fodder. United States guar splitting plants produce a high-protein germ fraction and a low-protein hull fraction. These two fractions are then recombined to create guar meal. In the U.S. GM sells for approximately 60% of the price of dehulled soybean meal and is most commonly used in cattle feedlot operations (K. Forbes, West Texas Guar, Brownfield, Texas, personal communication).

The crude protein content of GM varies from 35 to 47.5% on a dry matter (DM) basis (Ambegaokar et al., 1969), and Verma and McNab (1984) reported that about 88% of the crude protein content in guar meal is true protein. Methionine and lysine concentrations in GM are lower than those typically found in soybean meal, although it is rich in arginine (Verma and McNab, 1984). The gross energy of raw and autoclaved GM for poultry were reported as 4.837 and 4.861 kcal/g while the N-corrected ME values of raw and autoclaved GM were 2.005 and 2.069 kcal/g respectively (Nagpal et al., 1971).

Excessive concentrations of GM in poultry diets cause diarrhea, depressed growth rate and increased mortality in broilers (Sathe and Bose, 1962; Couch et al., 1967; Verma and McNab, 1982), and decreased egg production and feed efficiency of laying hens (Bakshi et al., 1964; Nagra et al., 1985; Patel and McGinnis, 1985). GM is an effective full-fed alternative to inducing molt in laying hens which are typically deprived of feed for a period of days in order to replenish reproductive capacity (Gutierrez et al., 2008).

Guar gum is a galactomannan polysaccharide consisting of a β-1→ 4 linked D-mannopyrannose backbone with branched α-1→ 6 D-galactopyrannose side chains occurring every 2-3 mannose moieties (Choct, 1997). Residual gum content of typical guar meals can be as high as 18 to 20% (Bakshi et al., 1965), and a series of feeding experiments conducted by Vohra and Kratzer (1964) demonstrated that as little as 1% guar gum in broiler chicken diets causes growth depression, while a diet containing 2% guar gum resulted in broiler growth rates ranging from 61 to 67.4% that of the control group. However, dietary guar gum also has putative protective effects against colonization and subsequent proliferation by pathogenic bacteria, preventing microbial translocation within the GI tract (Bengmark, 1998) and reproductive tracts of laying hens (Gutierrez et al., 2008).

**Prebiotic Properties of Guar Gum**

Guar gum is readily fermented by human fecal microbes and it has bifidogenic effects (Okubo et al., 1994) which may partially contribute to its prebiotic functions. Although the effects of intact guar gum on gut microbial ecosystems have not been studied extensively, many investigations have demonstrated prebiotic functions of partially hydrolyzed guar gum (PHGG). In an in vivo experiment by Okubo et al. (1994), healthy adult human subjects were fed 21 g/d PHGG for 14 d to investigate the effects of PHGG intake on fecal microbiota, bacterial metabolites, and pH. The total viable counts of *Bifidobacterium spp*. and *Lactobacillus spp*. and the percentage of these species in the total count increased significantly during the PHGG intake periods. Fecal pH and fecal bacterial metabolites such as β-glucuronidase, putrefactive products and ammonia

content were also significantly decreased by PHGG intake. Ishihara et al. (2000) found that the cecal *Bifidobacterium spp.* and *Lactobacillus spp.* of pullets challenged with *Salmonella enteritidis* (SE) administered PHGG at 0.025% were significantly higher than those consuming diets without PHGG. The numbers of SE incidence within internal organs and intestine of pullets were also significantly decreased by the 0.025% PHGG treatment. The decrease in SE incidence was attributed to increased excretion of SE in feces. In a similar experiment, Ishihara et al. (2000) observed decreased SE present in internal organs and egg shell surface in groups fed diets containing 0.025% PHGG. Interestingly, higher concentrations of PHGG (0.05 and 0.1%) did not exhibit the same SE inhibition effect as 0.025% PHGG did.

**Anti-nutritive Compounds Contained in GM**

A limit of 2.5% GM included into broiler diets does not affect performance parameters of growing broilers (Lee et al., 2003). At higher levels of inclusion, depressed growth rates, poor feed efficiency, diarrhea, and increased mortality are reported (Patel et al., 1980). These effects were originally thought to be due to the presence of several anti-nutritional factors, namely residual guar gum and trypsin inhibitors.

Protease inhibitors are anti-nutrients commonly found in legumes which reduce effective activity of enzymes involved in amino acid digestion. Their potency depends primarily on their target enzyme (i.e., the placement in which the proteolytic cascade is inhibited). Soybeans, for example contain two groups of protease inhibitors, the large K unit which inhibits trypsin, and the smaller Bowman-Birk unit which inhibits trypsin and

chymotrypsin (Krogdahl et al., 1994). Due to the inhibition of trypsin, protease activation is decreased, thereby reducing the enzymatic digestion of amino acids. The presence of trypsin inhibitor also results in increased pancreatic secretion, which results in hypertrophy of the pancreas. This occurs as a result of an interruption in the regulatory feedback loop which controls pancreatic secretive activity (Francis et al., 2001). This results in an increased requirement for sulfur-containing amino acids, as pancreatic enzymes contain high levels of cystine (Krogdahl et al., 1994).

Bakshi et al. (1964) proposed that guar meal contains two deleterious factors responsible for the production decreases observed when feeding chickens, trypsin inhibitor and guar gum residue. The trypsin inhibitor was listed as a deleterious factor because the chicks fed guar meal had been reported to present pancreatic hypertrophy (Couch et al., 1967) which can also be found in chickens fed raw soybeans or soybean meal. However, trypsin inhibitor was not universally accepted as a primary factor for the deleterious effects of feeding guar products to poultry (Anderson and Warnick, 1964; Vohra and Kratzer, 1964). Verma and McNab (1982) reported that neither heating guar meal directly or steam pelleting diets containing guar meal had an effect on the performance of the broiler chicks. This agrees with the findings of Nagpal et al. (1971), who reported that autoclaving guar meal did not improve its gross protein value for chicks. Trypsin inhibitor activity in guar meal was significantly lower than in heat-treated soybean meal commonly used in poultry feed (Conner, 2002), which indicates that negative effects on performance of poultry when fed diets containing guar meal are not primarily due to trypsin inhibition.

In addition to protease inhibitors, GM contains approximately 4% saponins as reported by Kakani et al. (2007). Saponins are naturally occurring phytochemicals that consist of a fat-soluble nucleus, either steroid or triterpenoid in nature, with one or more water-soluble carbohydrate side-chains consisting of glucose, galactose, xylose, rhamnose, or glucuronic acid. When added in the diet, some saponins increase intestinal epithelial cell permeability, and have recently been suggested to be a culprit in the production decreases associated with GM (Gee and Johnson, 1988). In addition to increased cell porosity, this permeability results from a decrease in the transmembrane electrochemical gradient that is critical to active nutrient transport. It is thought that glycoside side chains play an important role in affecting this activity since soy saponins, which contain a straight tri-saccharide side chain has little effect in rat intestinal cells while guar and lucerne saponins, which contain branched tetra-saccharide glycone moieties cause significant changes in transmembrane potential differences (Oleszek et al., 1994).

In addition to changes in epithelial cell membranes, saponins obstruct absorption of some micronutrients. Increased excretion of iron and magnesium and decreased levels of plasma calcium and zinc have been observed in pigs fed high levels of lucerne saponins (Southon et al., 1988). Although a specific mechanism has not been proved, stereochemical binding to these minerals while in the digestive tract is thought to be the reason for decreased absorption rates. Formation of indigestible saponin-protein complexes are reported in diets containing saponins (Potter et al., 1993). This may

explain the relatively low digestibility rates observed in many legume seeds, which tend to contain high concentrations of saponins.

Generally, it seems that saponins contain antibacterial activity against Gram-positive bacteria rather than Gram-negative genera (Wallace et al., 1994). This tendency of anti-Gram-positive bacterial activity is similar to that of the ionophores, although their specific modes of action are different. Further, saponins seem to elicit a concentration-dependent growth response of in vitro bacterial cultures, with growth promotion occurring at low saponin levels and inhibition occurring at higher concentrations (Sen et al., 1998). Similarly, Hassan et al. (2007) reported that saponin extracted from GM elicited antibiotic activity against *Staphylococcus aureus* but not *Salmonella typhimurium* or *Escherichia coli*.

## ROLE OF THE CECA IN CARBOHYDRATE DIGESTION

### Physiology of the Ceca

Many researchers have speculated about avian cecal function. Evidence exists that the ceca play a role in the microbial degradation of carbohydrates (Jorgensen et al., 1996), absorption of water (McNab, 1973), cholesterol digestion and absorption (Tortuero et al., 1975), microbial synthesis of vitamins (Coates et al., 1968) and degradation of nitrogenous compounds (Goldstein, 1989). It is logical to assume that during the evolution of avian species, adaptation to environment and available nutrient supply influenced the type and structure of digestive system that developed. One must also consider that an organ with a blind sac such as the cecum would be susceptible to

parasitic invasion or infection, increasing selection pressure against the possession of that organ. Therefore, the fully developed avian ceca likely serve some important function although it has been established that the lack of a ceca does not negatively affect growth or performance in poultry (Beattie and Shrimpton, 1958).

The shape, size, and capacity of the ceca varies considerably among different avian species due to their evolutionary adaptation with some species abandoning the organ altogether. Stevens and Hume (1998) measured the relative lengths of intestinal segments in 644 specimens representing 24 orders, 51 families, 124 genera, and 166 species of birds, concluding that the most developed ceca were found in herbivores and granivores whose diet contains high levels of plant fiber or chitin, whereas the ceca of carnivores is generally underdeveloped or absent altogether. In the domestic fowl each cecum is 15-18 cm long and consists of a narrow, thickly walled open end and a slightly dilated, more thinly-walled blind portion (McNab, 1973). The ceca usually contain smooth-textured, dark brown odiferous material with a pH of approximately 6.5 - 7.0, though the nature of this material can be influenced by dietary factors (Halnan, 1949).

Thornburn and Willcox (1965) examined the part played by the ceca in carbohydrate digestibility. They found that overall digestibility of whole wheat, whole oats, whole barley, and a pelleted complete feed was reduced in cecectomized chickens compared to that of intact chickens. In contrast, starch digestibility was not negatively affected by cecectomy, indicating that the ceca may have a role in digestion of poorly digestible carbohydrates. This supports the findings of Radeff (1928), who found that neither cecectomized nor intact chickens were able to digest the crude fiber (CF) fraction

present in barley, but that intact chickens were better able to digest that of wheat (5.1%) than the cecectomized bird (1.4%). More pronounced differences were shown with the CF fraction of corn, in which the intact bird digested more (17.1%) than did the cecectomized chicken (0%).

**Microbial Ecology of the Ceca**

It is well recognized that the ceca are the site of the greatest concentration of intestinal microorganisms in mature chickens (Johansson et al., 1948), and characterization of the cecal microbiota in birds started in the early 1970s (Barnes et al., 1972). However, much less is known regarding the physiology of specific microorganisms found in the avian ceca compared with what is known about the microorganisms that inhabit the rumen. Only 10-60% of the bacteria in the ceca can be cultivated using anaerobic culture techniques (Zhu et al., 2002). The inability to grow these microorganisms in the laboratory limits the ability of researchers to elucidate specific biochemical and genetic details associated with the colonization of these organisms.

Over 200 different bacterial species have been isolated in the chicken, most of which are strict anaerobes (Barnes, 1979). These populations are labile and are influenced by factors such as diet, health status, and age. In the first days of life in the healthy chicken the predominant cecal bacteria are *Enertobacteriacae*, *Enterococcus*, and *Lactobacillus spp*., while *Bacteroides* and *Eubacterium* populations are established after 2 weeks (Van der Wielen et al., 2001). According to Mead (1989), *Lactobacillus spp.* in chickens mainly include *Lactobacillus acidophilus, Lactobacillus salivarius*, and *Lactobacillus*

*fermentum*, all of which appear in significant numbers ($10^8$-$10^{10}$ / g cecal contents) by day 4 of life and are considered to be probiotic in nature.

Recently, molecular techniques were used to examine the ecology of the cecal environment. These approaches have some limitations, namely the possibility that DNA isolation and amplification may be biased in favor of certain bacteria and genetic sequences. However, it is generally agreed that they provide a more accurate assessment of the microbial diversity present in the avian ceca than do traditional culturing techniques. The use of comparative 16S rDNA analysis has been used successfully by several researchers as a means of quantitating changes in bacterial populations resident in the ceca of chickens (Hume et al., 2003; Muyzer et al., 1993). In this procedure, bacterial DNA is isolated from chicken cecal contents, amplified using PCR techniques specific for the variable 16S region of bacterial DNA. These amplicons are then put through an electrophoretic process that utilizes a formamide and urea denaturing gradient as a means of determining differences in DNA base pair content, and thus differences in bacterial communities.

**Fermentative Activity of NSP within the Ceca**

Based on the diversity of the cecal microbial ecosystem, cecal microbes are able to utilize a variety of substrates commonly found in feedstuffs. In addition, cecal microbiota may also utilize some components not digested by the host animal. Even though the quantities of NSP are low in commercial poultry diets, specifically those which are corn-based, there are still soluble NSP (10-25 g/kg DM) present (Jamroz et al., 2001). Tsukahara and Ushida (2000) reported that feeding a plant protein-based diet to

chickens led to a higher production of SCFA than a diet based on animal protein. This difference is most likely due to a higher concentration of NSP components in the plant-based diet and perhaps a higher activity and density, or both, of the resident microbiota.

The major microbial activity in the avian ceca is fermentative rather than putrefactive, producing methane and carbon dioxide in approximately equal parts, hydrogen sulfide and ammonia (~1% of $CO_2$ concentration) to a lesser extent, and short-chain fatty acids (SCFA) on the order of parts per thousand that of $CO_2$ (Shrimpton, 1963). The short-chain organic acids that are produced during cecal fermentation are similar to those produced in the human large intestine or the rumen. These include acetate, propionate, butyrate, lactate, valerate, and isovalerate (Shrimpton, 1963).

As in the GI tract of other species, acetic acid is produced in the greatest quantity with lesser amounts of propionic and butyric acids and trace amounts of others, although this production can be slightly altered according to diet composition (Annison et al., 1968). Butyrate, a four-carbon short chain fatty acid (SCFA), is a prime energy substrate of the colonocyte, which provides 70% of the energy needed for maintenance and differentiation of the colonic epithelium in rats (Roediger, 1980). Butyrate enhances growth of the gut epithelium and promotes gut cell differentiation, improves immune functions of the host, induces apoptosis in colonic tumor cell lines and improves the intestinal barrier function (Brouns et al., 2002). After fermentation, protonated forms of SCFA are transported though cecal epithelial membranes. Morton (1978) concluded that the amount of energy obtained from these SCFA is too low to meet all energy requirements of poultry. However, some birds, such as the ostrich, may obtain as much

as 75% of their energy from this process. In the emu, carbohydrate fermentation may contribute up to 50% of the maintenance energy requirement (Herd and Dawson, 1984), similar to what is seen in ruminants.

Galactomannan residues in guar meal (guar gum) which contain the β -1→4 glycosidic bond that cannot be digested by humans and animals is well fermented by microbes in the GI tract, which produce high amounts of SCFA. Compared with other non-ruminants (i.e. pigs, rats), the cecal microbiota in chickens is less able to ferment NSP fractions (Carre et al., 1990; Jorgensen et al., 1996). Additionally, Langhout and Schutte (1996) suggested that not only the amount but also the type of NSP plays a role on cecal fermentation. Jamroz et al. (1998) demonstrated the role of species and time since last feeding as factors that affect cecal fermentation. This agrees with Prop and Vulnik (1992) who observed that day length and feeding frequency correlate with altered cecal fermentation of NSP.

In addition to energy-yielding activity, SCFA in the ceca of avian species can provide other benefits. Van der Wielen et al. (2000) demonstrated that high fermentation activity in chicken ceca was correlated with a lower pH which may inhibit pathogenic bacterial colonization (Russell, 1992). McHan and Shotts (1993) observed a toxic effect of SCFA to some *Enterobacteriacae*, and in vitro studies showed a 50-80% reduction in *Salmonella typhimurium* counts in the presence of SCFA. While SCFA have a bacteriostatic effect to some enteric bacteria and *S. typhimurium*, these organic acids do not inhibit beneficial GI tract bacterial such as *Lactobacillus spp.* (Van der Wielen et al., 2000).

**DIET PRE-CONDITIONING**

Changes in the gut size of gallinaceous birds as a function of diet, particularly in reference to fiber content in the diet were first reported by Farner (1960). In many species there is a seasonal variation in gut size, related to qualitative and quantitative factors in the diet which are related to providing for increased digestive efficiency. Among roughage-eating species of birds, such as grouse, gut and cecal sizes are smallest in summer when birds eat a variety of foods such as fruits and tender shoots of newly growing herbaceous plants. They are longest and heaviest in winter when the birds feed primarily on woody plants and leaves (Gasaway, 1976). Fenna and Boag (1974) showed that grouse with small, summer-adapted guts are unable to digest their food efficiently when abruptly changed to a winter, high-fiber diet. These birds exhibited weight loss and increased mortality rates, however within 2 months, they showed an increased ability to thrive on the high-fiber diet. Thus, there is a time dependent alteration that occurs in response to dietary changes.

The ability of the ceca and other organs of the digestive system to respond to changes in volume intake as a function of fiber content of the diet is expressed both as organ hypertrophy and production of SCFA (McBee, 1977). However, the size of the hypertrophied digestive tract as a function of body weight varies markedly among species and may be an indicator of the quality or type of diet being consumed (Moss, 1977). The low end of hypertrophic expression is held by waterfowl (~3%) and domestic fowl (4-5%) with various grouse species exhibiting ceca amounting to 20-24%

of body weight. Domestic turkeys (10-12%) and Japanese quail (14-17%) are intermediate to these groups in terms of cecal hypertrophic abilities (Jamroz et al., 1998). Gasaway (1976) showed that gut size of wild rock ptarmigan ranges from 2% of body weight in summer to 6% of body weight in the winter. Changes occurred in the weight of the gizzard and length of the intestine and ceca, and there was an increase in the weight of their contents. Fenna and Boag (1974) showed that stimulatory conditions caused an increase in intestinal length of 26% and an increase in cecal length of 44% in Japanese quail. Moss and Trenholm (1987) demonstrated changes in the gizzard, small intestine, ceca, and large intestine of 52%, 10%, 5%, and 10%, respectively in red grouse. Miller (1976) investigated similar responses in mallard ducks and found an increase in cecal size from 2.3% of body weight to 3.2% of body weight and an increase of 23% in the quantities of SCFA present in the ceca when these birds were switched from a commercial mash diet to one consisting of alfalfa pellets. This latter result may reflect inefficient SCFA absorption in these birds, since the concentration SCFA in grouse ceca are much lower.

The time required for this adaptive hypertrophy as determined experimentally appears to be about 10 weeks. Fenna and Boag (1974) utilized a preconditioning period of 8 weeks in Japanese quail and spruce grouse. Duke et al. (1984) utilized a period of 10 weeks with the domestic turkey, and Moss and Trenholm (1987) utilized a period of 9 weeks with red grouse. The latter showed that after 11 weeks of conditioning, birds were still continuing to show improvement in their ability to digest a bulky diet. Bedbury and Duke (1983) compared the cellulolytic capability of high-fiber fed and

low-fiber fed turkeys following 8 weeks of preconditioning and found the former to have larger gizzards and ceca as well as a greatly increased capacity for cellulose digestion by mixed bacterial cultures taken from these birds with significant differences in bacterial flora observed between the two groups. In a subsequent study, ceca of domestic turkeys were shown to undergo a 25% increase in size and a four- to five-fold increase in $^{14}C$-cellulose uptake when fed a high-fiber diet (Duke et al., 1984). Thus exposure to high-fiber diets causes increases in the overall size of the gut including the gizzard, intestine, and ceca.

The major effects of preconditioning have been described in several species. Quantity of food consumed affects overall digestive tract size, and food quality affects the histology and microbiota composition of the gut, the rate of SCFA production and presumably the ability to absorb them (Hanssen, 1979; Duke et al., 1984). In comparing wild-taken willow grouse to captively managed ones, Hanssen (1979) reported large villi and an epithelium consisting entirely of tall columnar epithelial cells in wild birds collected in both winter and summer. Captive birds had much shorter villi, heavy infiltrations of mononuclear cells in the stroma of the villi, and an abundance of goblet cells, indicative of inflammation. Additionally, overall gut length in captive birds was shorter than either summer or winter-taken wild grouse, and the microbiota of wild-taken birds consisted of large numbers of spirochetes, amoebas, flagellates and anaerobic bacteria. Captive birds had much higher numbers of bacteria overall, and a significant proportion of them were coliforms and enterococci, the latter of which were not found in the wild grouse (McBee and West, 1969).

A study by Moss and Trenholm (1987) showed a relationship between fiber content, intake volume, and energy demands of the bird. By providing different amounts of fiber as a supplement to the diet of captive grouse, they found a "threshold" for cecal response. Below this threshold, the grouse responded to modest increases in fiber content of their diet by increasing feeding rates, which induced a compensatory increase in gizzard size. Above the threshold, apparently at the point where the birds could not meet energy demands by simply eating more food, there was a significant increase in cecal size as the energy demands of the birds required "extraction" of more energy from the diluted diet. Birds were then able to maintain weight without further increases in food intake, exhibiting an ability to extract additional energy presumably from cecal fermentative activity. An increase in cellulose digestion as suggested by Duke et al. (1984) would be a likely component of that increased capability.

If the various avian species in which cecal size and response to high-fiber diets have been studied were ranked, we would obtain in order from the smallest response to the largest, waterfowl, domestic chicken, domestic turkey, quail, and various ptarmigan and grouse. This ranking may correlate with the adaptive strategies these various species have for meeting energy requirements in the face of seasonally varying food supplies. Waterfowl are capable of migrating long distances and therefore are not required to meet energy demands from geographically restricted and seasonally changing food sources. They can essentially provide themselves with abundant quantities of high quality food throughout the year. Chickens, which were derived from the jungle fowl, evolved in an environment where high quality food was abundant and

the tropical/semi-tropical environment did not impose severe seasonal demands for energy expenditure in the form of thermoregulation. Turkey and quail are non-migratory and are capable of surviving in temperate geographical zones in which quality changes seasonally. Accordingly, they have the capability for moderate cecal hypertrophy (Pulliainen, 1976). Ptarmigans and grouse are mostly non-migratory and regularly subsist on poor quality diets for a portion of each year, enduring extreme conditions of the high arctic winter in many cases. Thus they are adapted to consume large quantities of nutrient-dilute food to meet an extreme demand for energy.

It seems likely that the ceca and its microbiota have a critical role in nutrient digestion in wild birds. Although this role is less obvious in domesticated species which generally consume a more refined grain-based diet, it is reasonable to expect some level of physiological adaptation when administered a low-quality, bulky diet. If this accommodation is indeed observed to exist, it may have significant economic implications, particularly as it relates to increasing grain prices, decreasing availability of antibiotic growth promoters, and consumer pressures concerning animal well-being.

The objectives of the research presented in this paper is to determine the effect of diet preconditioning (acclimation) period on the digestibility and potential increased use of GM in poultry diets. Although it is generally accepted that chickens cannot efficiently digest ingredients rich in NSP, a preliminary experiment conducted in our laboratory suggests that given an adequate period of time, relative increases in energy utilization of GM may occur.

A series of experiments evaluating the response of single comb white leghorn cockerels which were preconditioned to diets containing relatively high levels of GM over a 21- d period were conducted over a period of several months. These trials focused around changes in the GI tract of chickens such as digestive organ size and weight as well as changes in microbiota that reside within the ceca and colon of the GI tract as indicators of increased digestive capacity of otherwise poorly digestible feed ingredients such as GM. The determination of which changes most significantly affect digestibility may be useful when the addition of inexpensive, albeit poorly digestible feed ingredients into the diets of longer-lived poultry species such as breeding stock, egg-laying strains of chickens and perhaps turkeys are considered.

# CHAPTER III

# EFFECTS OF GUAR MEAL ON INTESTINAL MORPHOLOGY AND DIGESTIVE ORGAN WEIGHTS OF ADULT COCKERELS

## INTRODUCTION

The weight of the gastrointestinal (GI) tract is highly correlated to body size. This relationship is not constant over the life of an animal and can be influenced by certain dietary components including fiber (Dowling et al., 1967; Levine et al., 1974). Within the broad classification of fiber exists a subcategory known as non-starch polysaccharides (NSP) or dietary fiber, which are defined as complex carbohydrates (excluding α-glucans, starch) that are found in foods and include cellulose, pectin, glucan, gum, mucilage, inulin, and chitin (Choct, 1997). Non-starch polysaccharides are not well-digested by endogenous digestive enzymes of most monogastric animals. Due to their poor digestibility, ingredients containing relatively high levels of NSP usually contain low levels of metabolizable energy (ME) despite otherwise normal levels of gross energy. Non-starch polysaccharide components are found in many cereal grains and in most oilseed meals and are most often associated with the hull and underlying aleurone layers of the seed or grain (Eastwood, 1992).

Although many types of carbohydrate compounds fall under the category of NSP, three groups are of particular importance in poultry nutrition. These are the β-glucans in barley, the arabinoxylans (or pentosans) in wheat and the raffinose group of oligosaccharides in soybeans. In water, most NSPs produce viscous solutions. Highly

viscous solutions have negative effects on digesta flow and interaction of substrates with endogenous enzyme systems. Generally, increases in digesta viscosity increase the thickness of the unstirred water layer adjacent to the mucosal villi. Consequently, reduced solubilization and uptake of most nutrients follows. Additionally, opportunity for digestive enzymes to act upon food substrates decreases despite a general increase in digesta retention time. There is also an increase in endogenous digestive secretions which contain large amounts of protein and lipids in the form of enzymes and bile salts, respectively (Ikegami et al., 1990). This represents a net loss of nutrients associated with the digestion and absorption of NSP.

Non-starch polysaccharides known to increase the viscosity of intestinal contents also are associated with decreased nutrient (glucose, amino acids and lipids) absorption rates in pigs and chickens (Blackburn and Johnson, 1981; Rainbird and Low, 1986). Possible explanations for these observations include decreased enzyme activity limiting the rate of nutrient digestion in the GI tract, possibly through the adsorption and immobilization of enzymes and nutrients by NSP molecules (Shah et al., 1986). Perhaps as a compensatory adaptive response to this reduction in nutrient availability, ingestion of NSP results in an enlargement of the organs of the digestive system, including the stomach, small intestine, large intestine, and pancreas of rats (Ikegami et al., 1990) and the gizzard, small intestine, pancreas, and ceca of birds (Redig, 1989). These changes are characterized by increased secretion of digestive fluids accompanied by decreased nutrient digestibility.

In digestibility studies investigating the utility of whole flaxseed in laying hen diets, sudden incorporation of 8-10% flaxseed in the diet resulted in gut transit problems, usually manifest as reduced feed intake and wet manure (Gonzalez-Esquerra and Leeson, 2000). However, these problems usually can be overcome by gradual introduction of flaxseed over several weeks (e.g. 4% for one week, 6% during the second week, followed by 8-10% during the third week). Duke et al. (1984) reported similar findings in domesticated turkeys conditioned to consuming high levels of cellulose over the course of 10 weeks as determined by $^{14}$C-cellulose uptake.

Diet composition also plays a role in GI epithelial tissue intactness. Yamauchi (2002) reported microscopic alteration in the intestinal mucosa of rats consuming even low amounts of dietary NSP. Iji (1999) found that guar gum and xanthan gum significantly increased crypt depth of both the jejunum and ileum, suggesting that NSP may promote GI tract turnover. Greater crypt depth generally indicates increased villus cell proliferation, and therefore increased nutrient absorptive capacity which improves overall health. However, Redig (1989) suggested that feeding relatively high levels of NSP to poultry impacts production as increased fermentation in the ceca may lead to cecal hypertrophy, creating a relative increase in maintenance energy requirement associated with the enlarged organs. Thus it is tempting to hypothesize that differences in GI tract size can affect performance in either a positive or negative manner depending upon the level at which NSP compounds can be effectively utilized by the animal and just how closely this energy “threshold” can be approached without being surpassed.

Guar (*Cyamopsis tetragonoloba*) is a drought-tolerant, annual legume grown for its high concentration of galactomannan gum which is used as a thickener in the food, cosmetics and pharmaceutical industries. Its by-product, guar meal, is an important animal fodder, containing between 35 and 45% crude protein (Ambegaokar et al., 1969). Guar gum is a galactomannan polysaccharide consisting of a β-1→ 4 linked D-mannopyrannose backbone with branched α-1→ 6 D-galactopyrannose side chains. Typical guar meals contain approximately 18 to 20% guar gum (Bakshi et al., 1965). Vohra and Kratzer (1964) demonstrated in a series of experiments that as little as 1% guar gum in growing broiler chicken diets depressed growth rate, with diarrhea, weight loss, and mortality occurring at higher levels of inclusion.

Based on a previous experiment evaluating the effect of diet preconditioning on the ME of GM in leghorn cockerels (see Figure 1-1), adult chickens are hypothesized to be capable of physiological adaption to diets rich in NSP similar to adaptation observed in other gallinaceous species. The purpose of this research was to examine GI morphological response of adult white Leghorn cockerels consuming diets containing different levels of GM over a period of 3 weeks.

## MATERIALS AND METHODS

### Experimental Design and General Procedure

A randomized complete block design was employed using a total of 28 white leghorn cockerels of similar BW. Birds were assigned to individual cages (60 cm x 60 cm x 60 cm) in a windowless, environmentally controlled room where they received 14

h of light daily. Birds were allowed to acclimate to their new environment for 7 days before the trial began. Birds were assigned one of four isocaloric, isonitrogenous dietary treatments (Table 3-1), consisting of a commercial-type corn-soy laying hen diet (Control), a laying hen diet containing 6% GM, a laying hen diet containing 12 % GM, and a laying hen diet containing 24% GM. Feed and water was available ad libitum for the duration of the trial period. Individual body weight and feed consumption were recorded weekly beginning on d 1 and ending on d 21.

**Organ Weights**

After 3 weeks on their respective dietary treatments all roosters were euthanized by cervical dislocation and necropsied. Absolute and relative organ weights were determined for the ventriculus (with proventriculus), small intestine (duodena, jejuna, and ilea), heart, liver (with gall bladder), spleen, and pancreas. Digestive tract contents were eliminated either by bisection (proventriculus/ventriculus) followed by a water rinse or by gentle finger stripping (small intestine). In addition, cecal length was determined in each bird.

**Histological Sample Preparation**

Prior to finger stripping and weight determination of the small intestine, a 3-cm cross-section was excised 2-cm anterior to the distal end of each of the three regions of the small intestine: duodenum, from the pylorus to the distal portion of the duodenal loop; jejunum, from the distal portion of the duodenal loop to Meckel's diverticulum; and ileum, from Meckel's diverticulum to the anterior portion of the ileocecal junction.

**TABLE 3-1. Experimental diets for gastrointestinal morphometric analysis[1].**

| Ingredients | Guar meal (%)[2] | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| | ---------- (%) ---------- | | | |
| Corn | 65.52 | 63.08 | 60.61 | 55.67 |
| Guar meal[3] | 0.00 | 6.00 | 12.00 | 24.00 |
| Dehulled soybean meal | 22.43 | 19.10 | 14.77 | 6.10 |
| DL-Methionine | 0.11 | 0.13 | 0.14 | 0.18 |
| L-Lysine HCl | -- | 0.05 | 0.10 | 0.20 |
| Fat (animal-vegetable blend) | 0.50 | 0.71 | 1.43 | 2.87 |
| Limestone | 8.58 | 8.58 | 8.59 | 8.59 |
| Mono-dicalcium $PO_4$ | 1.65 | 1.66 | 1.67 | 1.68 |
| Salt | 0.41 | 0.41 | 0.41 | 0.41 |
| Trace minerals[4] | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamins[5] | 0.25 | 0.25 | 0.25 | 0.25 |

[1] Calculated analysis of all diets was as follows: crude protein, 17.0%; metabolizable energy, 2,770 kcal/kg; calcium, 3.70%; available phosphorus, 0.45%; methionine, 0.38%; lysine, 0.86%; threonine, 0.57-0.60%; and tryptophan, 0.17-0.18%.

[2]Calculated crude fiber and guar gum content was as follows: 2.39, 0.00%, 0% GM diet; 2.85, 0.96%, 6% GM diet; 3.31, 1.92%, 12% GM diet; and 4.23, 3.84%, 24% GM diet

[3] See Appendix I for the nutrient matrix.

[4] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[5]Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin $D_3$, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 µg Vitamin $B_{12}$, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

Each cross-section was rinsed with isotonic saline (0.9% NaCl) to remove fecal material from the intestinal lumen, labeled on the serosal surface with indelible ink for identification purposes and fixed in 10% Neutral Buffered Formalin for 72 h. Longitudinal strips (~1 cm) were then excised from the approximate midpoint of each cross-section and placed in histological cassettes for paraffin embedding and slide preparation by the Texas Veterinary Medical Diagnostic Laboratory. Slides containing a longitudinal section (4 micron thickness) of each intestinal region for each bird stained with hematoxylin and eosin (H&E) and used to determine histological parameters described below.

**Morphological and Morphometric Evaluation**

Slides prepared from birds consuming differing levels of guar meal were used to evaluate epithelial injury as evidenced by destruction of the villus architecture using a Zeiss Axiophot microscope using ImagePro Plus, Media Cybermetrics version 4.1.0.9 (Silver Spring, MD). Changes in the mucosa were grouped into six grades where grade 0 represents normal mucosa and grades I to V indicate increasing damage to the surface epithelium (Figure 3-1) as described by Chiu et al. (1970). Briefly, grade I is characterized by the development of subepithelial space at the tip of the villus. The space is more extended in grade II with epithelial lifting also observed. Grade III shows extensive epithelial lifting down the sides of the villi with a few villus tips becoming denuded. In grade IV there is extensive denuding of the epithelium and exposure of the lamina propria to the intestinal lumen. Grade V is characterized by hemorrhage and ulceration of the mucosa. A total of 30 well-oriented jejunal villi were scored from each

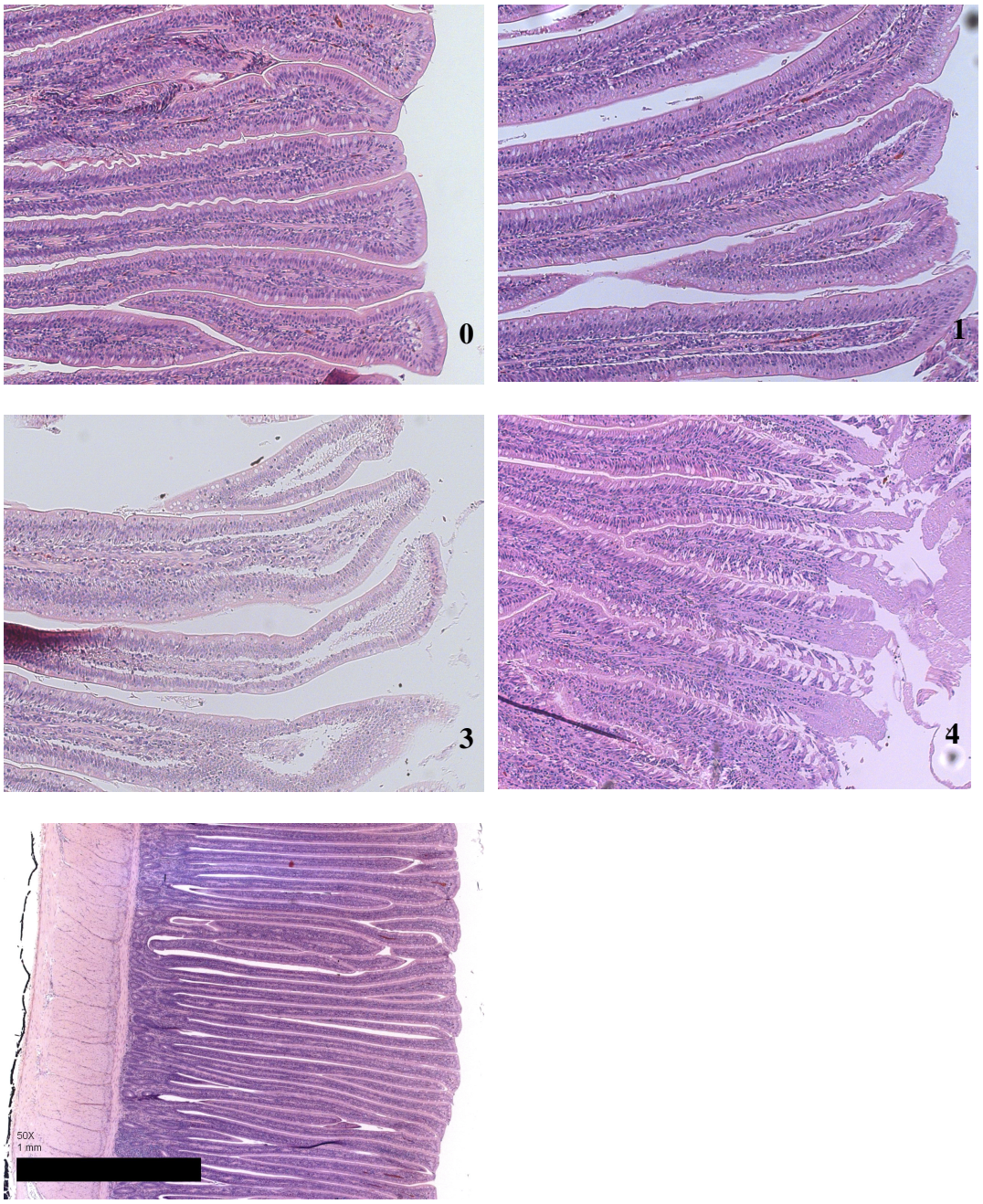


**FIGURE 3-1. Progression of intestinal mucosal lesions (grade 0, 1, 3, and 4) for comparative purposes (x 100) and villus height measurement (x 50).**

cockerel, yielding a total of 210 observations for each treatment group. Measurements of villus height were recorded for each intestinal region (identified by serosal dye color) of every bird. Villus height was calculated as the measurement from villus tip to muscularis mucosa (in microns) taken every 500 um over approximately two-thirds of the length of each intestinal fragment, yielding 12 measurements from each intestinal region per bird and 84 observations per treatment group.

**Statistical Procedures**

Body weight, feed consumption, relative organ weight and villus height data were evaluated as a randomized complete block design using the Mixed Models procedure of SPSS. Preconditioning diet was used as a fixed factor with block accounted for as a random factor and initial body weight included as a concomitant variable. Means were further separated by Duncan's multiple range test when appropriate. Intestinal lesion scores were analyzed by Kruskal-Wallis H and Mann-Whitney U non-parametric analyses. Statistical significance was accepted at $P \leq 0.05$.

## RESULTS

### Feed Consumption and Body Weight

After three weeks on their respective diets, significant differences in daily feed intake were not observed ($P < 0.06$) in cockerels consuming differing levels of GM (Table 3-2). However, a significant decrease in body weight was observed in the group consuming 24% dietary GM. Additionally, a significant difference in the change in

**TABLE 3-2. Feed intake, final body weight, and change in body weight in roosters after 3 wk of feeding diets containing 0, 6, 12 or 24% guar meal[1].**

| | Guar meal concentration (%) | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| Final Body Weight (g) | 1811 ± 97[a] | 1849 ± 153[a] | 1791 ± 122[a] | 1485 ± 231[b] |
| Daily Feed intake (g) | 128.3 ± 5.2 | 136.0 ± 5.3 | 134.0 ± 5.7 | 118.7 ± 5.5[†] |
| [2]Change in BW (%) | 2.09 ± 0.08[a] | 2.56 ± 0.06[a] | 2.01 ± 0.03[a] | -6.32 ± 0.13[b] |

[a-b] Means within a row lacking a common superscript are significantly different ($P < 0.05$).

[1] Mean ± SD, n = 7.

[2] Calculated as percent change in body weight from beginning to end of trial.

[†] Groups consuming 24% GM diet did not show statistically different means ($P < 0.06$).

body weight over the 3- week trial period was detected, with birds consuming the 24% GM diet losing weight whereas the other groups all gained weight at a similar rate.

**Organ Weights**

No significant differences in absolute or relative organ weights were observed in groups consuming up to 12% GM (Table 3-3). However, those consuming 24% GM had significant increases in the relative weight of the entire small intestine, liver, and pancreas. A numeric increase in the relative weight of the ventriculus (with proventriculus attached) and a numeric decrease in the relative weight of the spleen were noted, but were not considered to be statistically significant ($P < 0.78$). No significant differences were observed in absolute organ weights across the various dietary treatment groups. This suggests that the cause for significant changes in relative organ weights is in part due to significant differences in body weight (Table 3-3) rather than true change in organ size.

**Intestinal Morphology**

Cecum length was significantly increased in birds consuming 24% GM relative to those consuming lower levels (Table 3-4). Histological observations of the villus height of the duodenal and jejunal portions of the small intestine showed significant increases in the group consuming 24% GM relative to the other groups. Despite an apparent increase in overall intestinal surface area of birds consuming 24% GM, epithelial injury as described by Chiu et al. (1970), was significantly increased relative to the control group. The group consuming 6% GM showed a significant increase in epithelial damage relative to the control group as well, though it was intermediate to the

**TABLE 3-3. Absolute and relative organ weight of roosters after 3 wk of feeding diets containing 0, 6, 12 or 24% guar meal[1].**

| Organs | Guar meal concentration (%) | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| Proventriculus/Ventriculus | | | | |
| weight (g) | 39.7 ± 2.2 | 40.5 ± 5.5 | 37.4 ± 3.4 | 35.5 ± 6.5 |
| relative wt (%) | 2.20 ± 0.14 | 2.21 ± 0.33 | 2.11 ± 0.24 | 2.39 ± 0.22[†] |
| Small Intestine | | | | |
| weight (g) | 31.4 ± 3.3 | 39.7 ± 4.9 | 37.1 ± 2.9 | 48.9 ± 8.9 |
| relative wt (%) | 1.74 ± 0.25[b] | 2.16 ± 0.20[b] | 2.09 ± 0.15[b] | 3.29 ± 0.23[a] |
| Liver | | | | |
| weight (g) | 34.2 ± 2.4 | 37.8 ± 4.8 | 34.8 ± 3.1 | 35.7 ± 6.1 |
| relative wt (%) | 1.89 ± 0.09[b] | 2.05 ± 0.11[b] | 1.96 ± 0.25[b] | 2.42 ± 0.30[a] |
| Pancreas | | | | |
| weight (g) | 3.69 ± 0.19 | 3.97 ± 0.49 | 3.83 ± 0.42 | 4.01 ± 1.05 |
| relative wt (%) | 0.20 ± 0.01[b] | 0.22 ± 0.03[b] | 0.22 ± 0.02[b] | 0.27 ± 0.06[a] |
| Spleen | | | | |
| weight (g) | 3.96 ± 0.81 | 3.73 ± 0.84 | 3.75 ± 1.03 | 2.37 ± 0.84 |
| relative wt (%) | 0.22 ± 0.04 | 0.20 ± 0.04 | 0.21 ± 0.06 | 0.16 ± 0.04[†] |

[a-b] Means within a row lacking a common superscript are significantly different ($P < 0.05$).
[†] Groups consuming 24% GM diet did not show statistically different means ($P < 0.08$).
[1] Mean ± SD, n = 7.

**TABLE 3-4. Cecum length, villus height and intestinal epithelial lesion scores of roosters after 3 wk of feeding diets containing 0, 6, 12 or 24% guar meal[1].**

| Organs | Guar meal concentration (%) | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| Cecum Length (cm) | 13.9 ± 0.73[b] | 14.6 ± 0.73[b] | 14.3 ± 1.33[b] | 15.8 ± 1.15[a] |
| Epithelial Lesion Score | 1.2 ± 0.11[c] | 2.3 ± 0.12[b] | 1.1 ± 0.10[c] | 2.6 ± 0.11[a] |
| Villus Height (mm) | | | | |
| Duodenum | 1.84 ± 0.31[b] | 1.83 ± 0.31[b] | 1.96 ± 0.34[b] | 2.39 ± 0.22[a] |
| Jejunum | 1.68 ± 0.26[b] | 1.67 ± 0.34[b] | 1.77 ± 0.26[b] | 2.12 ± 0.27[a] |

[a-c] Means within a row lacking a common superscript are significantly different ($P < 0.05$).

[1] Mean ± SD, n = 7.

group consuming 24% GM. Measurements of villus height from the ileum were not analyzed due to inaccurate villus orientation along the microscope slide.

## DISCUSSION

The results from the present study indicate that the GM included into the diet at high levels (24%) affected the GI tract and associated organs, probably as a result of its high dietary fiber content. The most noticeable effect was on the small intestine, ceca, liver, pancreas, villus height, and intestinal lesion score. This is in agreement with Rubio and Brenes (1988) who reported that high amounts of indigestible materials in the digesta increased the length of the intestinal regions in chickens. Experiments with chickens, ducks, and geese showed that the amount and type of dietary fiber influenced the weights and lengths of the GI tract and associated organs (Jamroz et al., 2001). In a study by Borin et al. (2006), inclusion of 20% cassava leaf meal resulted in an increase in small intestine length and weight of 28.6% and 42.8%, respectively. Jorgensen et al. (1996) reported that high intake of NSP caused a significant expansion of the GI tract, and Jamroz et al. (2001) also stated that dietary fiber might be expected to influence the peristaltic activity and thereby the length and weight of the intestine.

In the current study the ceca length increased by more than 13% in birds consuming the 24% GM diet compared with to those consuming the 0% GM diet. In most gallinaceous species, the ceca are the major site of fiber digestion (Yu et al., 1998) and dietary NSP content is known to influence their weight and length, possibly because of the higher numbers of bacteria in the ceca on high fiber diets (Barnes, 1979). Gizzard

weights were not significantly different from each other, although a trend toward increased gizzard weight was observed in birds consuming the 24% GM diet ($P < 0.78$). The increase in gizzard size was probably related to the volume of the feed, increased time spent grinding the feed and increased frequency of gizzard contraction that is needed to digest and pass ingesta containing high levels of GM (Roche, 1981).

Non-starch polysaccharides also affect intestinal morphology (Iji, 1999). Increased duodenal and jejunal villus heights were noted in birds consuming the 24% GM diet when compared to birds consuming the 0% GM diet. Although slides were prepared from ileal tissue samples, an excessive number of sections were deemed unfit to measure accurately and were thus excluded from analysis. Intestinal villus heights in the present study were similar to those reported previously for adult Leghorn cockerels (Iji, 1999). Additionally, a trend was observed in which villus height was greatest in the duodenum followed by the jejunum which is in agreement with Yamauchi et al. (1995). These findings appear to be related to intestinal function, as vigorous nutrient absorption occurs in the upper small intestine.

As a result of feeding high levels of GM (24%), an increase in intestinal mucosal lesion score was observed. This damage may be caused indirectly by the viscous characteristics of NSP via a sloughing effect or an osmotic pressure effect (Stanogias and Pearce, 1985). Chiu et al. (1970) assumed that increased epithelial damage resulted in a two-fold nutrient loss due to a diminished capacity for optimal nutrient absorption across the brush border as well as a “healing” effect in which an influx of nutrients is required in order to replace damaged or dead cells. The extent of the nutrient demand

required for restitution of the epithelial lining in this experiment is not known. An increase in epithelial damage was also noted in birds consuming 6% GM but not 12% GM diets. It is not known whether this data is an artifact of the subjective nature of the scoring procedure or due to other factors since all data met the assumptions for analysis of variance (normality, independence and equal variances).

Although only one type of soluble NSP was evaluated, these results suggest that fermentable polysaccharides, especially those which affect intraluminal viscosity such as guar gum, can elicit significant morphological changes in the GI tract of chickens. The observed trophic effect of guar gum on the GI tissues/organs is in agreement with other researchers (Ikegami et al., 1990). These researchers suggest that the viscous nature of many soluble fibers result in increased fermentation products generated in the ceca and large intestine which in turn result in cecal or colonic hypertrophy (Edwards, 1993). However, evidence also exists which asserts that non-fermentable, non-viscous gums (Duke, 1986) lead to cecal hypertrophy in avian species as well. This suggests that the GI tract readily compensates for the impaired digestive processes associated with ingesting high volumes of poorly digestible substances, such as NSP by increasing its capacity for digestive fluid secretions as well as its overall absorptive surface area.

# CHAPTER IV

# EFFECT OF DIET PRE-CONDITIONING ON TRUE METABOLIZABLE ENERGY CONTENT OF GUAR MEAL IN ADULT COCKERELS

## INTRODUCTION

Supplying adequate energy to poultry is one of the most important aspects of a successful feeding program, and a great deal of research has been conducted in an attempt to obtain accurate data describing the energy value of feedstuffs for poultry (Hill and Anderson, 1958; Sibbald et al., 1963; Sibbald, 1976). The use of less costly, alternative feed ingredients to supplement or substitute conventional feed ingredients in poultry diets can be attractive economically, though many of these ingredients contain a broad range of anti-nutritive compounds, including non-starch polysaccharides (NSP) among others (Francis et al, 2002). Non-starch polysaccharides include a wide variety of carbohydrate moieties excluding α-glucans and are generally not digestible by endogenous avian digestive enzymes. Typically they are reported to act as anti-nutrients, negatively impacting animal growth and performance when included into the diet at moderate to high levels (Stanogias and Pearce, 1985). Diets and feed ingredients containing high levels of NSP also decrease voluntary feed consumption and nutrient digestibility, though these mechanisms are poorly understood.

Data on the interactions among energy-yielding nutrients are critical for estimating the metabolizable energy (ME) content of feed ingredients and diets for

experimental purposes. Metabolizable energy can be defined as a measure of the energy available to birds from their diet (Vohra, 1966), and although the gross energy content of a diet is relatively simple to determine, estimating its metabolizable energy is somewhat difficult. This is due to the fact that ME may not necessarily be additive from the caloric values of fats, proteins, and carbohydrates present in the diet (Sell et al., 1979).

Metabolizable energy can be expressed as either apparent metabolizable energy (AME) or as true metabolizable energy (TME). Apparent ME is defined as the gross energy of the food minus the energy lost as feces, urine and combustible gases when that foodstuff is consumed (Harris, 1966) with no direct correction for endogenous energy losses (EEL) inherent to the digestive and metabolic processes. True ME however, directly takes EEL into account usually through the measurement of excretory losses of fasted animals under identical experimental conditions, thereby increasing theoretical accuracy of the ME assay as a whole (Sibbald, 1976).

Managing the negative effects of NSP on animal performance requires an understanding of the physical and chemical characteristics of NSP and the physiological changes that occur in chickens when consuming these fractions. In diets containing readily digested feed ingredients, such as corn, sorghum, and even wheat, traditional ME assays have proven useful and more importantly, reliable in terms of predicting the energy content of a given feedstuff or complete diet (McNab, 1989). However, several studies evaluating the energy content of diets containing substantial amounts of NSP-rich ingredients result in a rather large range of predicted AME content, presumably due to the various types and amounts of fiber sources (Livesey, 1990).

Furthermore, inclusion of fiber in the diet results in digestive organ hypertrophy, further complicating the matter of accurately predicting ME in diets containing substantial amounts of NSP. Increases in the proportional size of the GI tract result in increases in nutrient demand of these tissues and reduce the overall rate of energy utilization of the bird (Miller and Reinecke, 1986). In many gallinaceous species, seasonal changes in gut size directly relate to differences in diet, particularly fiber content (Farner, 1960).

Generally, non-captive, gallinaceous birds consuming highly digestible foods such as seeds, berries, and fruits tend to have "normal" sized GI tracts. However, as the seasons change, the diet transitions to a less-dense bulky diet containing increased amounts of roughage. As a result, the GI tract accommodates this period of relative hardship by increasing in size, thereby increasing its surface area (Gasaway, 1976). It is assumed that this response occurs in order to increase the likelihood of "extracting" and absorbing as much nutrition from its available food source as is possible. Other experiments showed that birds conditioned to a highly digestible summer-type diet were unable to efficiently utilize a poorly digestible winter-type diet if the diet was switched abruptly, indicating a time-dependent response (Fenna and Boag, 1974). In other words, if allowed a period of several weeks, and some cases months, many galliforms are capable of being conditioned to consume and efficiently utilize poorly digestible, high-fiber diets.

The adaptive process observed in grouse and ptarmigan is not surprising given the extreme environments these birds to inhabit. However, domesticated species, such

as turkeys and quail also are capable of such physiological changes (Pulliainen, 1976). Experiments evaluating the ability of the domestic fowl to accommodate poorly digestible diets by "gut compensation" were for the most part abandoned in the mid 1980s due presumably to a vast majority of results indicating an extremely limited capacity to increase fiber digestion despite the use of a diet preconditioning period. One should note, however, that all studies conducted on chickens evaluated their ability to adapt to a diet rich in insoluble fibers, primarily cellulose which is composed of β - 1→4 glycosidic linkages and is considered to be truly indigestible by monogastric animals (Carre et al., 1990).

Changes in the animal feeds market have made the use of non-traditional, often less expensive feed ingredients more probable than in recent history. These include increases in feed and grain costs coupled with advancements in exogenous dietary enzyme production technologies. Guar meal is a product of gum isolation from the guar (*Cyamopsis tetragonoloba)* bean. It contains between 35 and 45% protein and has commercial value as a livestock fodder (Bakshi, 1966). Historically, guar meal was considered to be anti-nutritive when included in monogastric animal diets due primarily to the presence of residual guar gum and saponin. A study by Thakur and Pradhan (1975) reports an inverse relationship between broiler performance and guar meal, when included at 0, 7.5, and 15% of the diet. Similarly, Lee et al. (2005) reported negative growth response in broilers fed GM at levels in excess of 2.5%, presumably due to excessive residual guar gum content. Laying hens are more tolerant of GM, consuming up to 5% dietary GM without ill effect on BW gain or egg production (Gutierrez et al.,

2007), possibly indicating that prolonged periods of feeding to adult birds results in an increased ability to utilize GM.

The purpose of this experiment was to determine the nitrogen-corrected true metabolizable energy (TMEn) of GM in adult leghorn cockerels following a preconditioning period of 3 wk. During this preconditioning phase, birds were assigned either control (0%) or 24% GM diets in accordance with findings from a preliminary experiment indicating an increase in energy utilization occurring after approximately 21 d while consuming a diet containing 20% GM (See Figure 1-1).

## MATERIALS AND METHODS

### Experimental Design and General Procedures

A partial cross-over experimental design was employed in the present study, which involved a series of three individual TMEn trial periods. Each trial period consisted of a diet preconditioning period, in which birds were allowed to acclimate or become "preconditioned" to one of two isocaloric, isonitrogenous diets, containing either 0 or 24% guar meal (Table 4-1) for a period of 3 weeks. The arrangement of treatments is displayed in Table 4-2a with a schedule schematic displayed in Figure 4-1. The same birds were used in each of the three respective trial periods. Following each 3-wk preconditioning period, a TMEn bioassay was conducted according to the methods of McNab and Blair (1988) with the exception that each bird served as its own negative control, rather than using a separate negative control group of birds as suggested by Sibbald (1976). A total of 28 adult White Leghorn cockerels were individually housed

**TABLE 4-1. Experimental diets for three-phase TMEn trial[1].**

| Ingredients | Guar meal (%)[2] | |
|---|---|---|
| | 0 | 24 |
| | ------- (%) -------- | |
| Corn | 65.52 | 55.67 |
| Guar meal[3] | 0.00 | 24.00 |
| Dehulled soybean meal | 22.43 | 6.10 |
| DL-Methionine | 0.11 | 0.18 |
| L-Lysine HCl | -- | 0.20 |
| Fat (animal-vegetable blend) | 0.50 | 2.87 |
| Limestone | 8.58 | 8.59 |
| Mono-dicalcium $PO_4$ | 1.65 | 1.68 |
| Salt | 0.41 | 0.41 |
| Trace minerals[4] | 0.05 | 0.05 |
| Vitamins[5] | 0.25 | 0.25 |

[1] Calculated analysis of all diets was as follows: crude protein, 17.0%; metabolizable energy, 2,770 kcal/kg; calcium, 3.70%; available phosphorus, 0.45%; methionine, 0.38%; lysine, 0.86%; threonine, 0.57-0.60%; and tryptophan, 0.17-0.18%.

[2]Calculated crude fiber and guar gum content was as follows: 2.39, 0.00%, 0% GM diet; and 4.23, 3.84%, 24% GM diet

[3] See Appendix I for the nutrient matrix.

[4] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[5]Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin $D_3$, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 µg Vitamin $B_{12}$, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

**TABLE 4-2. Diet assignment and excreta collection schedules for determination of TMEn for GM.**

**a)** Pre-conditioning diet assignment for partial cross-over experimental design.

| Trial period[1] | Bird group[2] | |
|---|---|---|
| | 1 | 2 |
| 1 | Control | Control |
| 2 | 24% GM | Control |
| 3 | Control | 24% GM |

[1] Each trial period lasted a total of 49 days.
[2] Each group consisted of 14 adult single comb white leghorn cockerels.

**b)** Feeding and excreta collection schedule within each trial period.

| Time | Excreta Collection Phase | |
|---|---|---|
| | Endogenous Excreta | Test Ingredient Excreta |
| 06:00 h Monday | Food withdrawn | Food withdrawn |
| 14:00 h Monday | 60 ml glucose (30 g) solution fed by tube | 60 ml glucose (30 g) solution fed by tube |
| 06:00 h Tuesday | 60 ml glucose (30 g) solution fed by tube | 60 ml glucose (30 g) solution fed by tube |
| 18:00 h Tuesday | 60 ml glucose (30 g) solution fed by tube | 90 g guar meal (30 g) slurry fed by tube |
| | Trays inserted | Trays inserted |
| 06:00 h Thursday | Excreta collected and oven-dried | Excreta collected and oven-dried |

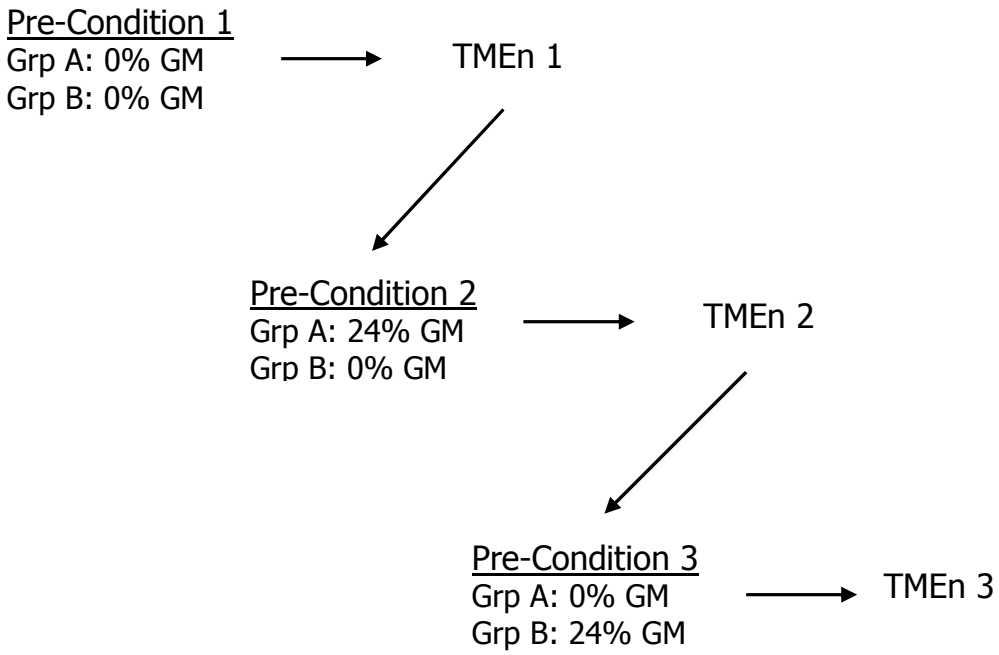


**FIGURE 4-1**. **Schematic of preconditioning diet assignment and general assay schedule for three-phase TMEn trial.**

in wire cages (60 cm x 60 cm x 60 cm) in a windowless room where they received 14 h of light daily. Water was available at all times and birds were fed ad libitum during each diet preconditioning period and its respective rest period. Body weight was recorded for each bird on d 1 and d 21 of each diet preconditioning period.

**Feeding Methodology**

Two tube feeding apparatuses were developed for use in this experiment. One device consisted of a 60-mL luer-tip syringe, to which a 20-cm section of Nalgene ™ tubing (6 mm O.D.) was attached to facilitate delivery of the dextrose solution directly into the birds' crop. The other device, used for administering the guar meal slurry, resembled a household sealant tube and consisted of an aluminum cylinder with a removable plastic end cap. This device was fitted with a 25-cm section of Nalgene ™ tubing (12 mm O.D.) for administration of the guar meal directly into the crop.

Table 4-2b illustrates the TMEn bioassay schedule used to determine endogenous energy losses (EEL) and test ingredient excreta energy for each trial period. Each respective trial period lasted a total of 49 days (21 d preconditioning period, 3 d EEL assay phase, 18 d rest phase, 3 d test ingredient phase, 4 d rest phase) after which their diet assignment would change.

Following each 21 d diet preconditioning period, birds were deprived of feed for 36 hours. At 8 and 24 h after feed withdrawal, all birds were tube-fed 30 g of glucose (dissolved into 60 mL of distilled water) and were allowed to void the contents of their GI tracts for a period of 36 h. At 36 h after feed withdrawal, all roosters were tube-fed 30 g of glucose solution (1 g glucose : 2 mL water) in order to determine EEL and

endogenous nitrogen loss for each bird. Following endogenous excreta collection, birds were allowed a "rest period" of 18 days during which water and the previously assigned preconditioning feed were available ad libitum. Following the 18-d rest period, all birds were subjected to the bioassay procedure described above with the exception that following the 36 h "purge period" in which GI contents were voided, cockerels were tube-fed approximately 30 g of guar meal which had been combined with water (1 g GM : 3 mL water) to form a slurry, in order to determine the true metabolizable energy of guar meal on each bird.

During both the endogenous excreta and the test ingredient excreta collection, stainless steel collection trays lined with parchment paper were placed under each cockerel for a period of 36 h. The total volume of excreta was collected, taking care to exclude inclusion of feathers, scale, and other debris from the collected sample. Upon completion of the test ingredient portion of the assay, birds were given a 4-day rest period in which birds were administered the previously assigned diet before advancing to the subsequent trial period.

**Sample Analysis**

All excreta samples were frozen (-20$^{o}$ C) immediately after collection. After completion of the experiment, all samples were thawed, dried at 85 C for 24 h in a forced draft drying oven as it was determined to be equivalent to lyophilization with respect to calorie retention within the sample in a preliminary experiment (data not shown) and ground through a 1- mm screen prior to analysis. Just prior to bomb calorimetry, dry matter content for guar meal and excreta samples was determined by

drying the samples at 105 C for 24 h in a forced draft drying oven. Energy content of the guar meal and excreta was determined by bomb calorimetry in a Parr Adiabatic (Moline, Illinois) bomb calorimeter. Nitrogen content of the guar meal and excreta samples was determined by combustion method using a Vario Elementar (Hanau, Germany) C/N analyzer.

**Statistical Procedures**

Data were subjected to a repeated measures analysis using the Mixed Models procedure of SPSS. Preconditioning diet was used as a fixed factor in determining changes in true metabolizable energy of guar meal, energy content of excreta, N content of excreta, and changes in body weight relative to baseline assessments which were determined during trial period 1. Statistical significance was accepted at $P \leq 0.05$.

**RESULTS**

Trial period 1 (Table 4-3) served as a baseline assessment period in that both groups of birds were pre-conditioned to the basal diet (0% GM) for a period of 3 wk followed by a TMEn assessment of GM. All data obtained during this baseline period was consistent, with only minimal bird-to-bird variation observed. When pre-conditioned to the basal diet, birds gained weight over the 3-wk period. As would be expected in fed and fasted animals, N and energy excretion was greater when birds were tube-fed rather than fasted. This observation is consistent across all trial periods.

During trial period 2, the group preconditioned to the basal diet consumed a similar amount of feed on a daily basis as was observed during the baseline period (trial

**TABLE 4-3. Final body weight, feed intake, nitrogen output, and metabolizable energy content of GM cockerels preconditioned to diets containing either 0 or 24% GM[1].**

| | Trial 1 | | Trial 2 | | Trial 3 | |
|---|---|---|---|---|---|---|
| | Preconditioning Diet | | Preconditioning Diet | | Preconditioning Diet | |
| | 0% GM | 24% GM | 0% GM | 24% GM | 0% GM | 24% GM |
| Final Body Weight (g) | 1864 ± 133 | -- | 1875 ± 154 | 1721 ± 178 | 1887 ± 191 | 1772 ± 129 |
| Daily Feed Intake (g) | 132.6 ± 3.8$^{a}$ | -- | 130.3 ± 4.1$^{a}$ | 117 ± 6.7$^{b}$ | 127.9 ± 4.9$^{a}$ | 115 ± 7.1$^{b}$ |
| Change in BW (%) | 1.19 ± 0.05$^{a}$ | -- | 1.14 ± 0.08$^{a}$ | -0.28 ± 0.09$^{b}$ | 0.13 ± 0.06$^{ab}$ | -0.32 ± 0.07$^{b}$ |
| AMEn (kcal/g DM) | 3.17 ± 0.17$^{a}$ | -- | 3.19 ± 0.27$^{a}$ | 2.78 ± 0.25$^{b}$ | 2.96 ± 0.19$^{ab}$ | 2.68 ± 0.25$^{b}$ |
| TMEn (kcal/g DM) | 3.47 ± 0.14$^{a}$ | -- | 3.50 ± 0.32$^{a}$ | 3.08 ± 0.28$^{b}$ | 3.18 ± 0.24$^{ab}$ | 2.97 ± 0.30$^{b}$ |
| Apparent Dig (%) | 69.2 ± 4.5$^{a}$ | -- | 68.3 ± 4.9$^{a}$ | 62.8 ± 7.0$^{b}$ | 63.6 ± 8.4$^{ab}$ | 57.8 ± 9.4$^{b}$ |
| Fasting Period | | | | | | |
| Energy excretion (kcal) | 10.78 ± 1.88$^{a}$ | -- | 10.44 ± 2.31$^{a}$ | 10.69 ± 2.27$^{a}$ | 7.74 ± 2.81$^{b}$ | 10.48 ± 2.63$^{a}$ |
| N excretion (g) | 0.75 ± 0.21$^{a}$ | -- | 0.72 ± 0.18$^{a}$ | 0.71 ± 0.16$^{a}$ | 0.48 ± 0.10$^{b}$ | 0.71 ± 0.22$^{a}$ |
| Tube-fed Period | | | | | | |
| Energy excretion (kcal) | 37.40 ± 4.97$^{b}$ | -- | 36.61 ± 4.71$^{b}$ | 50.97 ± 8.05$^{a}$ | 43.07 ± 7.80$^{ab}$ | 55.63 ± 11.06$^{a}$ |
| N excretion (g) | 1.47 ± 0.32$^{b}$ | -- | 1.51 ± 0.30$^{b}$ | 1.91 ± 0.39$^{a}$ | 1.75 ± 0.58$^{ab}$ | 2.09 ± 0.54$^{a}$ |

$^{a-b}$ Means in the same row lacking of common superscript were significantly different ($P < 0.05$).
[1]Means ± SD (n = 14).

period 1), while birds pre-conditioned to the 24% GM consumed significantly less feed on a daily basis. This difference in feed intake resulted in a significant decrease in BW change in the 24% GM group over the 3-wk trial period. No significant differences in either energy or N excretion were noted during the feed-deprived period between the 0 and 24% GM pre-conditioned groups. However, following the tube-feeding portion of the assay, significant increases in energy and N excretion and significant decreases in energy utilization and apparent digestibility were observed in birds pre-conditioned to 24% GM relative to those pre-conditioned to the 0% GM diet during trial period 2 as well as those observed during the baseline period.

In trial period 3, a trend similar to that observed during trial period 2 was evident in that birds pre-conditioned to the 24% GM diet performed poorly relative to the baseline observations during trial period 1. Specifically, significant decreases in daily feed intake resulted in decreased BW in the 24% GM pre-conditioned group along with increases in energy and N excretion in both the feed-deprived and tube-fed conditions. Additionally, a significant decrease in the TMEn and apparent digestibility of GM was observed in this group. During this trial period, birds pre-conditioned to the control diet did not respond as those observed during trial period 2 in that their performance was intermediate to those preconditioned to the 24% GM diet in periods 2 and 3, and those pre-conditioned to the control diet during periods 1 and 2. This resulted in several non-significant differences occurring during trial period 3 between birds conditioned to the basal diet and the 24% GM diet although clear trends were evident. It should be noted that previous exposure to the 24% GM diet and probable injury to the intestinal

epithelium during the second trial period (Table 4-2a) is the most likely explanation for this observation.

## DISCUSSION

The ME value of a feedstuff is a function of its combustible energy content and digestibility. Since the combustible energy of fiber is relatively consistent, changes in digestibility will affect the ME value (Livesey, 1990). The TME concept described by Sibbald (1976) is based on the assumptions that fecal metabolic and endogenous urinary energy excretions remain constant regardless of feed intake. However, this may not be the case when consuming indigestible or poorly digestible feedstuffs. In a study by Sibbald and Price (1980) evaluating the variability of endogenous energy losses in cockerels consuming 5% dietary alfalfa meal, the authors attain greater assay accuracy when applying the self-corrected EEL than when using the group-corrected EEL, yet state that its use may not be necessary due to the additional labor and cost associated with such changes in the protocol. In a separate study, Sibbald (1980) evaluated endogenous energy and nitrogen losses associated with consuming diets containing high levels of cellulose and sawdust. In this report, the author found that the addition of these indigestible materials did not affect endogenous energy loss or nitrogen excretion. It should be noted however, that both cellulose and sawdust are insoluble and are considered to be biologically inert, while many soluble fibers stimulate significant physiological activity in the bowel, even when ingested over a short period of time (Noack et al., 1998; Fernandez et al., 2002).

The TMEn assay used in the current experiment followed the techniques devised by Sibbald (1976) and the modifications suggested by McNab and Blair (1988). The initial 36-h period of feed deprivation used in this study was intermediate to that suggested by Sibbald (1976) and by McNab and Blair (1988) in accordance with evidence that feedstuffs containing relatively high amounts of fiber remain in the GI tract longer than lower-fiber ingredients (Sibbald, 1979). Additionally McNab and Blair (1988) reported that the feed residue remaining in the digestive tract of cockerels after 48 h was determined to be significantly less than that which remained after 24 h of feed deprivation (0.17 ± 0.08 and 1.59 ± 0.56 g, respectively) which further supported the purge period of 36 h in this assay.

Feeding of dextrose to birds during the time period in which endogenous energy losses are collected decreases the rate of weight loss as well as the variability of endogenous losses, thereby providing a more acceptable means of energy determination with respect to animal well-being (McNab and Blair, 1988). It presumes that 100% of the dextrose is absorbed and therefore does not affect endogenous energy losses. One criticism against the use of dextrose during a total collection-type TMEn assay would be associated with the risk of spillage or regurgitation of the solution onto the collection vessel and subsequent misrepresentation of gross energy values. Mohamed et al. (1986) suggested the necessity to feed the test material in two portions to avoid regurgitation. This was not observed when feeding approximately 35 g GM, although it was noted that resistance was consistently encountered at an esophageal depth of ~8 cm during feeding tube intubation. If delivery of the ingredient bolus occurred prior to this restrictive point

in the esophagus, apparent ingredient regurgitation would occur. If however the feeding tube passed beyond this point (i.e. directly into the crop) a significantly larger bolus of feed could be delivered without subsequent regurgitation.

In general, most observations in the present study concerning endogenous energy and nitrogen losses, change in body weight, and excreta volume were similar to previously reported values. In experiments with adult cockerels, fasting energy losses of 9 to 10 kcal/24 hr and 12 to 15 kcal/48 h were reported by Tenesaca and Sell (1981) and Yalcin and Onol (1994). These values are similar to 10.78 kcal/36 h observed in the baseline assessment period of the current study (Table 4-3). A large portion of this energy was due to excretion of undigested residues derived directly from gastric, pancreatic and intestinal secretions that persist even when chickens are fasted. Nitrogen excretion in feed-deprived birds was similar across trial periods, with the exception of birds consuming the 0% GM diet during trial period 3 (Table 4-3). This agrees with the observation of decreased gross energy output in that group during the same trial period. This is likely due to a recuperative-type affect in which birds that had previously consumed the 24% GM diet were regaining body weight and likely restoring damaged GI epithelial tissues. Chiu et al. (1970) reported a similar observation in rats subjected to intestinal ischemia.

The increase in energy excretion in tube-fed birds preconditioned to the 24% GM diet can be expected for a number of reasons. Firstly, the presence of material, food or otherwise stimulates gastric and pancreatic secretions (Collip, 1922) resulting in increased energy excretion. Also, the degree of excretion of sloughed GI tissues is

proportional to DM intake and passage through the GI tract (Sturkie, 1976). Severe intestinal epithelial damage may have limited the absorptive capacity of birds previously consuming the 24% GM diet. This is evidenced by consistent increases in energy and nitrogen excretion coupled with decreases in apparent digestibility and metabolizable energy coefficients (Table 4-3). Correction of TME for nitrogen resulted in a 2 to 4% reduction in TME values of ingredients examined in both experiments, which is consistent to the reduction reported by McNab and Blair (1988) of approximately 2.8 to 5% for various vegetable-based protein sources. Furthermore, TMEn values obtained in the current experiment were 9-10% higher than AMEn values, which agree with previous reports of a 9 to 18% difference between TME and AME values in a variety of feed ingredients (Sibbald and Price, 1977).

With respect to determining the TMEn value of GM in poultry, cockerels which underwent the conventional bioassay, in which no preconditioning period was utilized, exhibited TMEn values of approximately 3.50 Kcal/g on a DM basis while those which were preconditioned to GM exhibited values in the range of 3.15 to 3.20 Kcal/g on DM basis. This is substantially different from the previously reported value of 2.01 Kcal/g (Nagpal et al., 1971) and actually closer to values for highly digestible ingredients such as corn (3.47 Kcal/g) and sorghum (3.38 Kcal/g) (NRC, 1994). Regrettably, an internal standard in the form of soybean meal or another well-accepted ingredient was not incorporated into the design of this experiment, nullifying the possibility of scaling values up or down as appropriate.

The practical implications of these results are not perfectly clear due to the lack of dietary inclusion levels of GM intermediate to 0 and 24% during the preconditioning phase of the trial. However, the severe decrease in apparent digestibility and energy utilization of GM at high levels of inclusion, suggests a lack of nutrient additivity. Sibbald (1977) showed that various combinations of corn, wheat, soybean meal, and tallow yielded TME values that reflected additivity of the TME value of individual ingredients. However, information concerning the influence that high levels of fibrous ingredients have on additivity of TME among poultry feedstuffs is lacking. The current study indicates that the passage of undigested material through the GI tract also increases the excretion of fecal metabolic energy. As a result, metabolic and endogenous energy excretion would not necessarily remain constant regardless of feed intake, but could vary depending upon the digestibility of the dry matter consumed.

# CHAPTER V

# EFFECT OF DIET PRE-CONDITIONING ON ENDOGENOUS ENERGY LOSSES IN ADULT COCKERELS

## INTRODUCTION

Metabolizable energy (ME) is a measure of the energy available to birds from their diet, and over the last three decades has become universally accepted as the preferred measure of the energy content of poultry diets (McNab, 1999). Metabolizable energy can be expressed as either apparent (AME) or true (TME) metabolizable energy. AME is defined as the gross energy content of the food minus the energy lost as feces, urine and combustible gases when that foodstuff is consumed (Harris, 1966). The energy metabolized is considered to be "apparent" because, of the energy excreted when the food is consumed, only part of it has been derived directly from the food. That is, a portion of the excreta emanates directly from the bird and is known as the endogenous energy loss (EEL). Part of the EEL is of fecal origin and is considered to consist of gut epithelial tissue, bile excretions and unabsorbed enzymes, while part is urinary and consists primarily of the excretory products of nitrogen metabolism (Sibbald, 1976).

Energy values based on AME bioassays have been widely used and form the basis of many nutritionists' data banks. However, many problems have been encountered with regards to the reliability of the AME assay. Among them, highly variable values for the same feedstuff as a result of differing bird age and strain (Rao and Clandinin, 1970); level of feed intake (Guillaume and Summers, 1970); presence of non-

starch polysaccharides (NSP) (Verma, 1977); and length of time in which birds were acclimatized to the diet/feedstuff prior to assay (Lodhi et al., 1969). This variability was ultimately attributed to the contribution made to the excreted energy by the EEL.

It was Sibbald (1976) who developed a bioassay which determined the TME of feedstuffs and accounted for the variability of EEL by measuring it directly in a group of birds separate from those undergoing the tube-feeding portion of the assay. In this procedure, a group of cockerels is starved for a period of time sufficient to void any residual intestinal contents, after which its excreta is collected for a duration of time identical to that of its tube-fed counterparts, usually 24 to 48 h (McNab and Blair, 1988). This ultimately provided a more direct measure of energy availability than did the traditional AME bioassay with lower incidence of highly variable results.

The introduction of the rapid bioassay proposed by Sibbald (1976) and its subsequent modifications (Sibbald, 1979; McNab and Blair, 1988) have resulted in a reproducible, relatively easy to manage program which can be conducted in many laboratory settings evaluating highly digestible ingredients. However, the assumption that EEL in the fasted group of birds is equal to that of birds undergoing the test ingredient portion of the assay has not been thoroughly investigated with ingredients containing substantial amounts of NSP. One possibility for addressing differences between group-derived EEL values would include the use of each individual animal as its own negative control. This would allow for a more precise determination of EEL since differences in metabolic activity unique to each animal would be accounted for directly.

A handful of studies have addressed this concern with ingredients such as alfalfa (Sibbald and Price, 1980) as well as non-combustible colloidal ingredients such as silica (Tenesaca and Sell, 1981), both of which found that the use of "self-controlled" EEL values resulted in more accurate, albeit more resource-demanding, predictions of TME content. No studies have addressed the validity of this assumption with ingredients that contain soluble forms of NSP. Non-starch polysaccharides encompass a wide variety of carbohydrate compounds excluding α-glucan (starch) polysaccharides. The presence of NSP in the diet can be a limiting factor when formulating monogastric animal feeds. This is due to the fact that NSP molecules cannot be digested by endogenous monogastric dietary enzymes, resulting in decreased digestibility and nutrient utilization (Choct and Annison, 1992).

It is widely recognized that ingredients which contain substantial levels of NSP (e.g. barley and rye) are far less digestible and contain lower levels of ME than ingredients which contain relatively low levels of NSP (e.g. rice, corn, and sorghum). Inclusion of ingredients rich in NSP increase intestinal viscosity and interact with the morphology and microbiota of the GI tract (Choct, 1997). These modifications to the physiology of the GI tract typically result in changes in digestive enzyme and fluid secretion volumes, which may affect maintenance requirements in birds consuming these ingredients (McNab, 1973).

Guar meal contains up to 18% of residual guar gum (GG), which is a soluble galactomannan polysaccharide composed of β-1 → 4 linked mannopyrannose backbone with branched α-1 → 6 galactopyrannose side chains. It is generally considered to be

poorly digestible for most monogastric animals and has negative effects on production parameters of poultry when included at levels greater than 1% of the diet (Vohra and Kratzer, 1964). The AME content of GM is 2005 kcal/kg as determined by Nagpal et al. (1971) though it has been observed in our laboratory that this value is extremely difficult to replicate in that significantly different (usually higher) values can be achieved, depending upon which method is used to determine TME. The purpose of this experiment is to examine the variation of EEL in birds consuming different levels of guar meal (GM), and to determine whether employing a "self-corrected" EEL measurement provides greater accuracy with respect to predicting the nitrogen corrected true Metabolizable energy (TMEn) content of GM than does the more typical "group-corrected" EEL term described by Sibbald (1976).

## MATERIALS AND METHODS

### Experimental Design and General Procedures

This experiment consisted of a cross-over design involving two TME trial periods, each consisting of a 3-wk diet preconditioning period in which birds were allowed to acclimate to one of three isocaloric, isonitrogenous diets, containing 0, 6, or 12% GM (TABLE 5-1). Following each 3-wk preconditioning period, a TMEn bioassay was conducted according to the methods of McNab and Blair (1988). Each trial period consisted of two phases, an EEL collection phase in which birds were unfed, and a tube-fed test ingredient phase to determine the TME of GM based upon either "self-," or "group-," corrected EEL values.

Two different sets of TME values were determined using estimates of EEL calculated as, 1) a "group-corrected" value, in which the EEL of all birds within a given treatment were pooled and served as a common EEL term irrespective of time, and 2) a "self-corrected" value, in which the TME of GM for each fed bird was determined by correcting for the EEL of that same bird during its respective unfed cross-over period. Essentially, the group-correction corresponds to the traditional TME assay suggested by Sibbald (1976), and is the typical procedure reported in the vast majority of ME studies, while the self-correction is similar to that suggested by Sibbald and Price (1980) which has been shown to be of limited benefit in evaluating ingredients containing large volumes of insoluble NSP (20% alfalfa).

A total of 30 adult White Leghorn cockerels were individually housed in wire cages (60 cm x 60 cm x 60 cm) in a windowless room where they received 14 h of light daily. Water was available at all times and birds were fed ad libitum during each diet preconditioning period and its respective rest periods. Body weight was recorded for each bird on d 1 and d 21 of each diet preconditioning period. Three dietary treatments (0, 6, and 12% GM) were randomly assigned to ten birds which were subdivided into two groups for a period of 3 weeks. Nitrogen-corrected TME of GM and EEL for each bird was determined according to methods of McNab and Blair (1988) which utilizes supplemental glucose, administered during the extended fasting portions of the TMEn bioassay to limit excreta variability as it provides a minimal level of the birds' daily energy requirement and does not result in measurable increases in EEL since bioavailability is considered to be 100%.

**TABLE 5-1. Experimental diets for cross-over TMEn assay[1].**

| Ingredients | Guar meal (%)[2] | | |
|---|---|---|---|
| | 0 | 6 | 12 |
| | ---------- (%) ---------- | | |
| Corn | 65.52 | 63.08 | 60.61 |
| Guar meal[3] | 0.00 | 6.00 | 12.00 |
| Dehulled soybean meal | 22.43 | 19.10 | 14.77 |
| DL-Methionine | 0.11 | 0.13 | 0.14 |
| L-Lysine HCl | -- | 0.05 | 0.10 |
| Fat (animal-vegetable blend) | 0.50 | 0.71 | 1.43 |
| Limestone | 8.58 | 8.58 | 8.59 |
| Mono-dicalcium $PO_4$ | 1.65 | 1.66 | 1.67 |
| Salt | 0.41 | 0.41 | 0.41 |
| Trace minerals[4] | 0.05 | 0.05 | 0.05 |
| Vitamins[5] | 0.25 | 0.25 | 0.25 |

[1] Calculated analysis of all diets was as follows: crude protein, 17.0%; metabolizable energy, 2,770 kcal/kg; calcium, 3.70%; available phosphorus, 0.45%; methionine, 0.38%; lysine, 0.86%; threonine, 0.57-0.60%; and tryptophan, 0.17-0.18%.

[2]Calculated crude fiber and guar gum content was as follows: 2.39, 0.00%, 0% GM diet; 2.85, 0.96%, 6% GM diet; and 3.31, 1.92%, 12% GM diet.

[3] See Appendix I for the nutrient matrix.

[4] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[5]Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin $D_3$, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 µg Vitamin $B_{12}$, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

**Feeding Methodology**

Two tube feeding apparatuses were developed for use in this experiment (See Chapter IV). One device consisted of a 60 mL luer-tip syringe, to which a 20- cm section of Nalgene ™ tubing (6 mm O.D.) was attached to facilitate delivery of the dextrose solution directly into the birds' crop. The other device, used for administering the guar meal slurry, resembled a household sealant tube and consisted of an aluminum cylinder with a removable plastic end cap. This device was fitted with a 25- cm section of Nalgene ™ tubing (12 mm O.D.) for administration of the guar meal directly into the crop. Table 5-2a illustrates the TMEn bioassay schedule used to determine EEL and test ingredient energy excretion for each subgroup during its respective trial period. A general assay schematic is displayed in Figure 5-1. Each trial period lasted a total of 45 days (21 d preconditioning period, 3 d EEL or test ingredient phase, 18 d rest phase, 3 d EEL or test ingredient phase).

During each respective TMEn trial period, birds were allowed a 21-d diet preconditioning period, after which they were deprived of feed for 36 hours (Table 5-2b). At 8 and 24 h after feed withdrawal, all birds were tube-fed 30 g of glucose (dissolved into 60 mL of distilled water) and were allowed to void the contents of their GI tracts for a period of 36 h. At 36 h after feed withdrawal, all roosters were tube-fed 30 g of glucose solution (1 g glucose : 2 mL water) to determine EEL and endogenous nitrogen loss for each bird. Following endogenous excreta collection, birds were allowed a "rest period" of 18 days during which water and the previously assigned preconditioning diet were available ad libitum. Following the 18-d rest period, all birds

**TABLE 5-2. Diet assignment and excreta collection schedules for determination of endogenous energy loss (EEL) variability in birds consuming 0, 6, and 12% guar meal (GM) diets.**

**a)** Pre-conditioning diet assignment for cross-over experimental design.

| Trial period[1] | Bird group[2] | | | | | |
|---|---|---|---|---|---|---|
| | 1A | 1B | 2A | 2B | 3A | 3B |
| 1 | 0% EEL | 0% GM | 6% EEL | 6% GM | 12% EEL | 12% GM |
| 2 | 0% GM | 0% EEL | 6% GM | 6% EEL | 12% GM | 12% EEL |

[1] Each trial period lasted a total of 45 days.
[2] Each group consisted of 5 adult cockerels.

**b)** Feeding and excreta collection schedule within each trial period**.**

| Time | Excreta Collection Phase | |
|---|---|---|
| | Endogenous Excreta | Test Ingredient Excreta |
| 06:00 h Monday | Food withdrawn | Food withdrawn |
| 14:00 h Monday | 60 ml glucose (30 g) solution fed by tube | 60 ml glucose (30 g) solution fed by tube |
| 06:00 h Tuesday | 60 ml glucose (30 g) solution fed by tube | 60 ml glucose (30 g) solution fed by tube |
| 18:00 h Tuesday | 60 ml glucose (30 g) solution fed by tube | 90 g guar meal (30 g) slurry fed by tube |
| | Trays inserted | Trays inserted |
| 06:00 h Thursday | Excreta collected and oven-dried | Excreta collected and oven-dried |

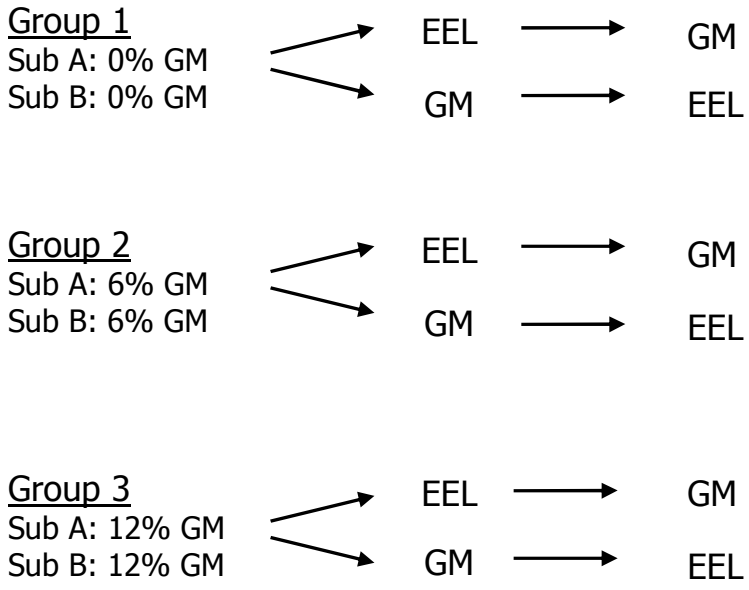


**FIGURE 5-1. Schematic of preconditioning diet assignment and general assay schedule for cross-over TMEn analysis.**

were subjected to the bioassay procedure described above with the exception that following the 36-h "purge period" in which GI contents were voided, cockerels were tube-fed approximately 30 g of guar meal which had been combined with water (1 g GM : 3 mL water) to form a slurry, in order to determine the true metabolizable energy of guar meal on each bird.

During both the endogenous excreta and the test ingredient excreta collection, stainless steel collection trays lined with parchment paper were placed under each cockerel for a period of 36 h. The total volume of excreta was collected, taking care to exclude inclusion of feathers, scale, and other debris from the collected sample.

**Sample Analysis**

All excreta samples were frozen (-20$^{o}$ C) immediately after collection. After completion of the experiment, all samples were thawed, dried at 85 C for 24 h in a forced draft drying oven as it was determined to be equivalent to lyophilization with respect to calorie retention within the sample in a preliminary experiment (data not shown) and ground through a 1- mm screen prior to analysis. Just prior to bomb calorimetry, dry matter content for guar meal and excreta samples was determined by drying the samples at 105 C for 24 h in a forced draft drying oven. Energy content of the guar meal and excreta was determined by bomb calorimetry in a Parr Adiabatic (Moline, Illinois) bomb calorimeter. Nitrogen content of the guar meal and excreta samples were determined by combustion method using a Vario Elementar (Hanau, Germany) C/N analyzer.

**Statistical Procedures**

Data were subjected to a repeated measures analysis using the Mixed Models procedure of SPSS. Preconditioning diet was used as a fixed factor with block accounted for as a random factor and initial body weight included as a concomitant variable for determining changes in nitrogen-corrected true metabolizable energy of guar meal, endogenous energy losses, and changes in body weight relative to group-corrected assessments. Means were further separated by Duncan's multiple range test when appropriate. Statistical significance was accepted at $P \leq 0.05$.

**RESULTS**

No significant differences in final body weight, change in body weight or daily feed intake were observed in the various treatment groups (Table 5-3). However, trends indicating an increase in feed intake and final body weight in birds consuming 6 and 12% dietary GM were noted relative to the control group. This supports the argument that previously reported energy values for GM may be underestimating its actual energy content (See Chapter IV).

The use of "self-corrected" EEL terms resulted in significant increases in the TMEn of GM across all treatment groups relative to those determined by the more commonly used "group-corrected" method (Table 5-4). When corrected using the group- corrected EEL, a significant decrease in the TMEn of GM was observed in birds preconditioned to the 6% GM diet relative to those preconditioned to the control diet, with the group preconditioned to the 12% GM diet intermediate to both groups.

**TABLE 5-3. Final body weight, feed intake and change in body weight in cockerels during cross-over TMEn assay[1].**

| | Guar meal concentration (%) | | |
|---|---|---|---|
| | 0 | 6 | 12 |
| Final Body Weight (g) | 1834 ± 46 | 1877 ± 130 | 1860 ± 123 |
| Daily Feed intake (g) | 127.8 ± 4.3 | 134.4 ± 7.6 | 133.2 ± 3.6 |
| [2]Change in BW (%) | 2.3 ± 0.06 | 2.5 ± 0.06 | 2.5 ± 0.07 |

[a-b] Means within a row lacking a common superscript are significantly different ($P < 0.05$).

[1] Mean ± SD, n = 10.

[2] Calculated as percent change in body weight from beginning to end of trial.

**TABLE 5-4. Group- and self-corrected TMEn values of GM and nitrogen and energy balances for cockerels preconditioned to 0, 6 and 12% dietary GM[1].**

| | Preconditioning Diet | | |
|---|---|---|---|
| | 0% GM | 6% GM | 12% GM |
| Group-Corrected | | | |
| TMEn (kcal/g DM) | $2.79 \pm 0.21^{a, x}$ | $2.62 \pm 0.47^{b, x}$ | $2.71 \pm 0.28^{ab, x}$ |
| Self-Corrected | | | |
| TMEn (kcal/g DM) | $3.12 \pm 0.15^{y}$ | $3.05 \pm 0.12^{y}$ | $3.08 \pm 0.11^{y}$ |
| Fasting Period | | | |
| Endogenous energy loss (kcal) | $14.64 \pm 3.87^{a}$ | $9.96 \pm 3.89^{b}$ | $10.59 \pm 3.83^{b}$ |
| N excretion (g) | $0.91 \pm 0.22^{a}$ | $0.76 \pm 0.15^{b}$ | $0.77 \pm 0.15^{b}$ |
| Tube-fed Period | | | |
| Endogenous energy loss (kcal) | $74.25 \pm 6.23^{a}$ | $69.73 \pm 5.71^{b}$ | $69.09 \pm 4.38^{b}$ |
| N excretion (g) | $1.66 \pm 0.31^{a}$ | $1.50 \pm 0.22^{b}$ | $1.52 \pm 0.23^{b}$ |

[a-b] Means in the same row lacking of common superscript were significantly different ($P < 0.05$).
[x-y] Means in the same column lacking of common superscript were significantly different ($P < 0.05$).
[1] Mean ± SD (n = 10).

When using the "self-corrected" method of determining TMEn of GM no significant differences were observed as a result of preconditioning diet. Additionally, significant changes in EEL and N excretion were observed as a result of preconditioning diet during both the fasting and tube-fed test ingredient portions of the assay (Table 5-4). During both the fasted and tube-fed periods of the assay, birds consuming 6 and 12% GM diets showed significant decreases in EEL and N excretion relative to birds consuming the control diet.

**DISCUSSION**

There are currently two ways in which EEL is commonly derived, 1) by measuring the excreta voided by a separate group of fasted birds or by birds given an energy source which is completely absorbed (e.g. glucose), and 2) by extrapolating to zero intake a line relating energy excretion to energy intake (McNab and Blair, 1988). This leaves users of TME assays to rely upon EEL values which are determined indirectly, in that different birds are used for each portion of the assay and should be considered to be an approximation at best.

One consideration regarding EEL variability and its affect on the accuracy of the TMEn bioassay is the potential for improvement by using each bird as its own negative control. That is, the EEL of each bird would be measured and used to correct the excreta energy output of the same bird when assaying a feedstuff. This would invariably lengthen the duration, cost, and work involved when undertaking these assays, as each animal would effectively undergo the TMEn bioassay twice. However, an increase in

precision would be expected as shown by Sibbald and Price (1980) in evaluating the TME content of alfalfa.

Fasting is the most widely applied method and is used and recommended by Sibbald (1976). However, while fasting, individual birds void variable amounts of energy depending upon the duration of the period. Values ranging from 6 to 16 Kcal/24 h (Sibbald and Price, 1980) and from 8 to 20 kcal/24 h (Farrell, 1978) are reported for EEL from cockerels following a 24- h fast. McNab and Fisher (1982) report greater variability (6 - 28 kcal/24 h) following a 48 h fast, although this is significantly reduced if birds are administered 25 g glucose on each of the 2 days in which the bird is being fasted (McNab and Blair, 1988). In experiments on adult cockerels, fasting energy losses of between 12 and 22 kcal per 48 h were reported (Yalcin and Onol, 1994), which are similar to the range of 10 to 15 kcal per 36 h observed in the present study. Correction of TME for nitrogen resulted in a 2 to 3% reduction in TME of GM, which is similar to the 2 to 5% reduction reported by Adeola et al. (1997) in ducks.

Studies on wild galliforms suggest that increasing fiber consumption increases overall feed intake as a compensatory response (Moss and Trenholm, 1987) to less energy-dense diets. This was not observed in the current investigation as consuming up to 12% dietary GM did not have an effect on final body weight, daily feed consumption, or change in body weight over the 21- d diet preconditioning period (Table 5-3). This finding is similar to observations in an earlier experiment in which only birds consuming extremely high levels of GM (24%) showed a negative correlation with body weight

gain and feed consumption rates relative to those consuming up to 12% dietary GM (See Chapter III).

Use of "group-" and "self-" EEL corrections resulted in significant differences in the TMEn of GM as a consequence of either self- or group- EEL correction terms were quite distinct across all treatment groups. Self-corrected TMEn values ranged between 11 and 12% higher than those calculated according to the group-EEL correction factor (Table 5-4). This is quite different than the 1-2% change in the TME of alfalfa as a result of self- and group- corrected terms report by Sibbald and Price (1980). Bird-to-bird variation for biologically active feed ingredients, such as those which contain high levels of soluble NSP, like GM is significant to the extent that it (perhaps erroneously) penalizes the TME value of the given ingredient. Additionally, the variation obtained from the self-corrected method of determining TMEn is substantially decreased, resulting in a more precise assay (SD = 0.12 and 0.11 for 6 and 12% GM self-corrected groups compared to 0.47 and 0.28 for conventionally-determined groups, respectively). This increase in precision may warrant the additional inputs, such as labor, time, resources and bird stress involved in its determination.

Preconditioning diet had a significant effect upon the EEL and N excretion of birds during both the fasting and the test-ingredient phases of the bioassay (Table 5-3). Birds preconditioned to the 6 and 12% GM diets showed consistently lower levels of EEL and N excretion relative to those consuming the 0% GM diet. Coupling this observation to an earlier study in which some degree of epithelial damage was observed as dietary GM increased (Table 3-4), it is assumed that these decreases in EEL and N

excretion can be attributed to recuperative-type effects in which the bird seems to retain some level of ingested energy and N in order to "heal" the tissues within the GI tract which may have been injured as a result of consuming elevated levels of GM. McCullough et al. (1998) reported similar epithelial injury occurring in rats consuming relatively high levels of NSP. However, rates of EEL and N excretion increased as NSP content increased in rats whereas the opposite response was observed in the present study.

Thus, it appears that a 21-d diet preconditioning period negatively impacted bird health in that TMEn of GM decreased significantly when calculated using the standard group-corrected method. Interestingly, use of the self-corrected EEL means of determining TMEn resulted in slight, non-significant differences as a result of preconditioning diet. Therefore, the use of the group-corrected TMEn value for GM is not recommended for this type of ingredient due to its reported affects on digestion, body and organ weight, and gut microbiota community. Additionally, if a group of birds is to be used to assay a series of feeds containing increasing levels of a particular ingredient, use of the self-correction would be appropriate in order to minimize bird to bird variation as a result of changing feeds and the birds' ability to better digest and utilize that feed over time.

# CHAPTER VI

# CECAL MICROBIOTA AND GROWTH PERFORMANCE IN ADULT COCKERELS FED DIETS CONTAINING GUAR MEAL

## INTRODUCTION

Studies on the composition of intestinal microbiota of chickens date back to 1901 (Rahner) and were continued in the 1940s (Shapiro and Sarles, 1949). However, comprehensive surveys that attempted to culture as many of the intestinal bacterial as possible were not carried out until the 1970s (Barnes et al., 1972; Salanitro et al., 1974; Mead and Adams, 1975). These studies are technically very difficult since strict anaerobic conditions have to be maintained during isolation and biochemical differentiation of the bacteria. Although other parts of the digestive tract of chickens might also be important sites for host-microbiota interactions, the ceca have received the most attention as it is considered to be very diverse yet relatively stable host site once established (Mead, 1997).

The study of intestinal microbiota composition has relied almost exclusively on the quantitative culture of microbes from fecal and intestinal tissue samples. Data from culture-based studies indicated that only between 10 and 60% of bacteria in the cecum successfully grew in culture (Barnes, 1979). Enumeration of particular microbial genera or species relies on the use of selective media. The inability to culture all microbes present in samples and the use of a limited range of reliable selective media introduces bias into analyses of the composition of normal microbiota.

More recently, relatively simple techniques have been developed to evaluate the complex ecosystem of the gastrointestinal (GI) tract of animals (Pryde et al., 1999; Netherwood et al., 1999). These methods involve the amplification by polymerase chain reactions (PCR) of 16S ribosomal DNA (rDNA) from microbial DNA extracted from samples collected from particular habitats. Amplified 16S rDNA sequences are cloned and should contain copies of the genes from all of species represented in the sample which can then be run through some form of gradient gel electrophoresis to determine ecological change in response to some stressor.

Denaturing gradient gel electrophoresis (DGGE) was first described as a useful technique to evaluate complex microbial populations by Muyzer et al. (1993). This method allows for visualization of PCR amplicons representing predominant intestinal bacterial communities by subjecting them to a gradient of chemical denaturant (urea and formamide) in parallel to the electric field used during typical electrophoretic procedures. Briefly, amplicon melting (separation of the double stranded DNA) domains and migration through the polyacrylamide gel denaturing gradient are determined by the unique guanosine + cytosine (G + C) content, sequence and interaction with associated bases (Fischer and Lerman, 1983). At the point in the gradient where partial denaturation of the DNA fragment occurs, its migration is severely retarded and sequences of the same size but of different chemical stability can be separated (Tannock, 2001). Because 16S rDNA from different bacterial species have different nucleotide base sequences in the variable region, they have different melting stabilities which result in distinct migration patterns through the gel. Differences in gene

primary sequences can be detected at the level of one base-pair substitution, and changes in the predominant microbial constituents are revealed by the presence or absence of amplicon bands (Murray et al., 1996).

Guar meal (GM) is the by-product of guar gum (GG) processing from the guar (*Cyamopsis tetragonoloba*) bean. It contains approximately 35% protein with a favorable amino acid profile and is generally priced considerably lower than other protein-rich oilseed meals. Due to its relatively high level of residual GG (~18%), it is used as livestock fodder in India and Pakistan with only limited use in the diets of monogastric animals. Guar gum is a fermentable, soluble polysaccharide comprised of a mannose backbone with galactose side chains occurring every two to three mannose moieties. Guar gum significantly affects the digestive process when consumed even in small amounts. These effects include increased intestinal viscosity, decreased absorptive capacity of the small intestine, modification of gut morphology, and interaction with the microbiota which normally reside within the GI tract of animals.

Previous work in our laboratory has indicated changes in the digestibility and rate of utilization of GM in cockerels which were acclimated or preconditioned to relatively high levels of GM. Although phenotypic changes were observed in the weight and size of the GI tract of these birds in response to increased dietary GM, the possibility of adaptation of the intestinal microbiota has not been evaluated. The purpose of this investigation was to determine the effect of increased intake of dietary GM on the cecal microbiota in adult cockerels.

## MATERIALS AND METHODS

### Experimental Design and General Procedure

A randomized complete block design was employed using a total of 24 adult white leghorn cockerels of similar BW. Birds were assigned to individual cages (60 cm x 60 cm x 60 cm) in a windowless, environmentally controlled room where they received 14 h of light daily. Birds were allowed to acclimate to their new environment for 7 days before the trial began. Birds were assigned one of three isocaloric, isonitrogenous dietary treatments (Table 6-1), consisting of a commercial-type corn-soy laying hen diet (Control), a laying hen diet containing 6% GM (6% GM), and a laying hen diet containing 12 % GM (12% GM). Approximately 0.5 g of cecal droppings, identified by their dark-colored, homogenous, pasty appearance were collected from a minimum of five birds from each respective treatment group once weekly. Sample collection took place in the morning (30–45 min after the lights came on) at which time the majority of birds voided their cecal contents with minimal contamination by urinary and fecal matter. Samples were collected on days 1, 8, 15, and 22 of the trial into 50-mL conical tubes and were immediately frozen (-80$^{o}$ C) for subsequent DNA isolation and amplification (below). Individual body weight and feed consumption were recorded weekly beginning on d 1 and ending on d 22. Feed and water was available ad libitum for the duration of the trial period.

### DNA Isolation and PCR

Genomic DNA was isolated from 1 mL of each sample with a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) using the method supplied by the manufacturer.

**TABLE 6-1. Experimental diets for cecal microbial population assay[1].**

| Ingredients | Guar meal (%)[2] | | |
|---|---|---|---|
| | 0 | 6 | 12 |
| | ---------- (%) ---------- | | |
| Corn | 65.52 | 63.08 | 60.61 |
| Guar meal[3] | 0.00 | 6.00 | 12.00 |
| Dehulled soybean meal | 22.43 | 19.10 | 14.77 |
| DL-Methionine | 0.11 | 0.13 | 0.14 |
| L-Lysine HCl | -- | 0.05 | 0.10 |
| Fat (animal-vegetable blend) | 0.50 | 0.71 | 1.43 |
| Limestone | 8.58 | 8.58 | 8.59 |
| Mono-dicalcium $PO_4$ | 1.65 | 1.66 | 1.67 |
| Salt | 0.41 | 0.41 | 0.41 |
| Trace minerals[4] | 0.05 | 0.05 | 0.05 |
| Vitamins[5] | 0.25 | 0.25 | 0.25 |

[1] Calculated analysis of all diets was as follows: crude protein, 17.0%; metabolizable energy, 2,770 kcal/kg; calcium, 3.70%; available phosphorus, 0.45%; methionine, 0.38%; lysine, 0.86%; threonine, 0.57-0.60%; and tryptophan, 0.17-0.18%.

[2]Calculated crude fiber and guar gum content was as follows: 2.39, 0.00%, 0% GM diet; 2.85, 0.96%, 6% GM diet; and 3.31, 1.92%, 12% GM diet.

[3] See Appendix I for the nutrient matrix.

[4] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[5]Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin $D_3$, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 µg Vitamin $B_{12}$, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

Samples were centrifuged for 10 min at 5,000 x *g* for 10 min. Each pellet was suspended in 180 µL of enzyme solution (20 mg/ml lysozyme, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton) for 30 min at 37° C.

PCR was conducted using the method of Hume et al. (2003). Bacteria-specific PCR primers to conserved regions flanking the variable V3 region of 16S rDNA genes were used. The primers (50 pmol of each per reaction mixture; primer 2, 5'-ATTACCGCGGCTGCTGG-3', and primer 3 with a 40-base GC clamp (Muyzer et al., 1993), 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCT ACGGGAGGCAGCAG-3') were mixed with Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO) according to the manufacturer's instructions, 250 ng of pooled (83 ng/replicate) template DNA from each of five replicates in each treatment group was added with 10 µg of bovine serum albumin to help stabilize the reaction. The PCR amplifications were performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). The program used was as follows: 1) denaturation at 94.9° C for 2 min; 2) denaturation at 94.0° C for 1 min; 3 annealing at 67° C for 45s, -0.5° C per cycle (touchdown to minimize spurious by-products (Don et al., 1991); 4) extension at 72° C for 2 min; 5) repeat steps 2 to 4 for 17 cycles; 6) denaturation at 94° C for 1 min; 7) annealing at 58° C for 45 sec; 8) repeat steps 6 to 7 for 12 cycles; 9) extension at 72° C for 30 min; 10) 4° C final.

**Gel Electrophoresis**

Denaturing gradient gel electrophoresis (DGGE) was run following the method of Hume et al. (2003) as modified from Muyzer et al. (1993). Amplicons were separated

on polyacrylamide gels (8% (v:v) acrylamide-bisacrylamide ratio 37.5:1, Bio-Rad, Richmond, CA) with a 35% to 60% urea deionized formamide gradient (100% denaturing 7M urea and 40% formamide) using a Dcode System (Bio-Rad, Hercules, CA). Amplified samples were mixed with an equal volume of 2X loading buffer (0.05% (w:v) bromophenol blue; 0.05% (w:v) xylene cyanol; and 70% (v:v) glycerol) and 4 μL were placed in each sample well (16-well comb). Gels were run at 60 volts for 17 h in 0.5X Tris-Acetate-EDTA buffer (TAE) (20 mM Tris (pH 7.4)); 10 mM sodium acetate; 0.5 M ethylenediaminetetraacetic acid (EDTA) at 59$^{o}$ C. Gels were stained for 30 min with SYBR Green I (USA Amersham Life Sciences, Cleveland, OH) diluted 1:10,000.

**Statistical Procedures**

Body weight and feed intake data were subjected to a one-way analysis of variance using diet as a fixed factor with block accounted for as a random factor and initial body weight included as a concomitant variable. Means were further separated by Duncan's multiple range test when appropriate. Statistical significance was accepted at $P \leq 0.05$.

The fragment analysis pattern relatedness was determined with Molecular Analysis Fingerprinting software (v 1.6; Bio-Rad, Hercules, CA). This analysis is based on the Dice similarity coefficient and the unweighted pair group method using arithmetic averages (UPGMA) for clustering. Comparisons between sample band patterns were expressed as a percent similarity coefficient (SC).

**RESULTS**

No significant differences in final body weight, change in body weight or daily feed intake were observed during this experiment (Table 6-2). Differences in cecal microbial ecology were noted in all three treatment groups in response to time exposed to their respective experimental diets. Birds consuming the control diet (0% GM) exhibited a slight change in microbial population occurring between wk 1 and 2 (87% SC). Between wk 2 and 3 however, a much higher degree of similarity (92.6%) was observed (Figure 6-1). In birds consuming 6% and 12% GM diets, more drastic differences were noted in response to diet acclimation period. For birds consuming the 6% GM diet, a 75% coefficient of similarity was observed between wk 1 and 2, which increased to 95.5% in the subsequent week. Similarly, birds consuming the 12% GM diet exhibited only a 59.8% SC between wk 1 and 2. This increased to 96.2% similarity between wk 2 and 3 (Figure 6-1).

When treatment groups are compared against one another over the course of the experiment, both diet and time had significant effects on the cecal microbiota of cockerels consuming GM diets. At wk 1 (Figure 6-2), birds consuming 6 and 12% GM diets share a 93.5% SC which is vastly different from birds consuming the control diet (63.7% SC). However, by wk 2 the control and 6% GM groups increase in microbial population similarity (93.0%), while the 12% GM group drops to a 83.3% SC , a trend which continues through wk 3 (91.1 and 84.0%, respectively) as shown in Figure 6-2.

Within each treatment group, a general change in microbial population profile was evident during the first week of the trial. By the end of the second week, however,

**TABLE 6-2. Final body weight, feed intake and change in body weight in cockerels during cecal microbial population assay[1].**

| | Guar meal concentration (%) | | |
|---|---|---|---|
| | 0 | 6 | 12 |
| Final Body Weight (g) | 1891 ± 113 | 1896 ± 121 | 1884 ± 107 |
| Daily Feed intake (g) | 129.3 ± 9.2 | 131.0 ± 8.2 | 131.7 ± 6.4 |
| [2]Change in BW (%) | 1.8 ± 0.03 | 1.7 ± 0.05 | 1.7 ± 0.06 |

[1] Mean ± SD, n = 8.
[2] Calculated as percent change in body weight from beginning to end of trial.

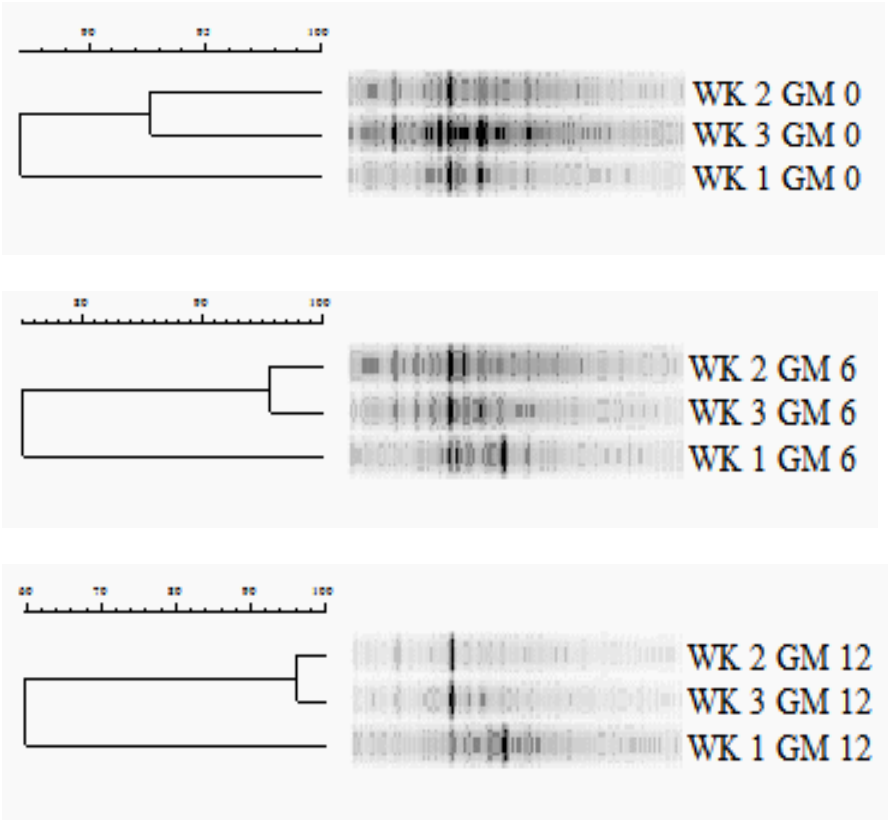


**FIGURE 6-1. Denaturing gradient gel electrophoresis of bacterial 16S rDNA amplicons band patterns from adult cockerels consuming 0, 6, and 12% GM diets broken out by time. The bar above figure indicates percentage similarity coefficients.**

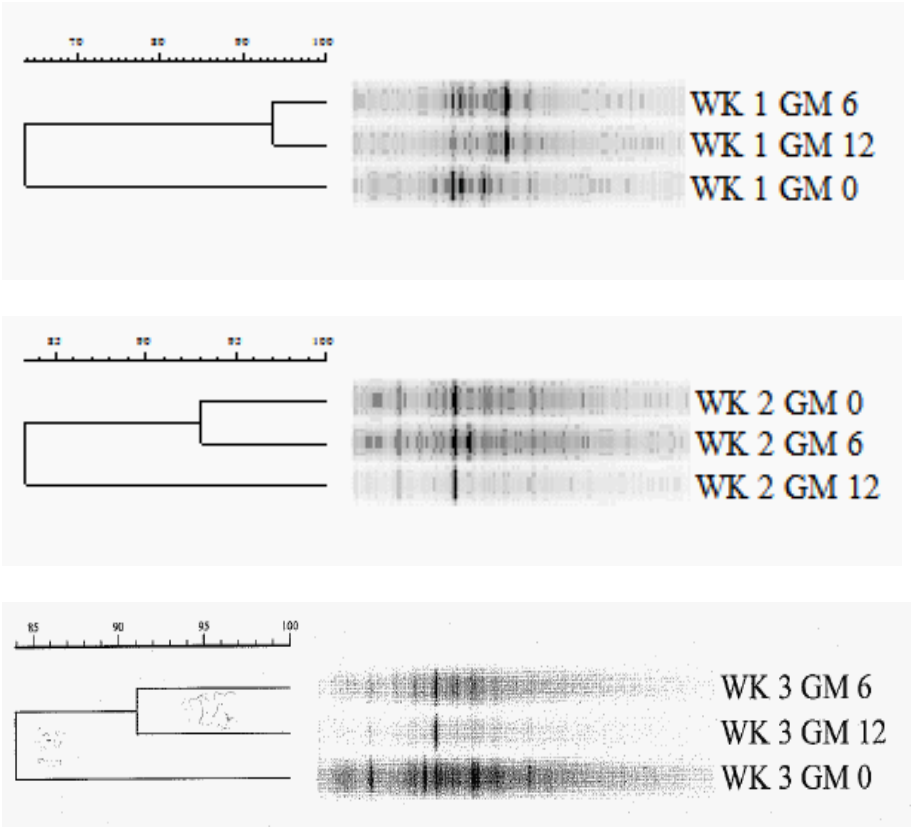


**FIGURE 6-2. Denaturing gradient gel electrophoresis of bacterial 16S rDNA amplicons band patterns from adult cockerels over a three week period of time broken out by GM treatment group. The bar above figure indicates percentage similarity coefficients.**

all treatment groups indicate a high level of similarity (> 93%). Furthermore, a difference in the cecal microbiota of birds consuming the 12% GM diet relative to those consuming the 0 and 6% GM diet becomes evident at this time (82.9% SC) indicating that following a stabilization period of 1-2 wk, differences in microbial ecological profile can be detected in birds consuming GM diets (Figure 6-3).

**DISCUSSION**

Microbiota in the mucosa of the GI tract are influenced by biochemical processes occurring in digesta and by factors originating from the animal host. Production and utilization of organic acids (acetic, propionic, butyric and lactic acids) in the ceca are described by Barnes et al. (1979). Furthermore, the degradation of uric acid ingressing into the cecum also is proposed based on the consistent observation of uric acid degrading bacteria among cecal isolates (Mead, 1974).

Knowledge of bacterial metabolism in the cecum is still incomplete, but fermentative processes appear to play a prominent role (Zhu et al., 2002). Some studies indicate that microbiota of the ceca in adult animals is relatively stable and that changes in the diet (from low- to high-protein diets for example) have little influence on microbial populations of the cecum (Takahashi et al., 1995). However, several types of dietary fiber have been reported to possess prebiotic functions which result in changes in the microbial community of the GI tract (Ishihara et al., 2000). Prebiotics are defined by Gibson and Roberfroid (1995) as, “nondigestible food ingredient which beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism

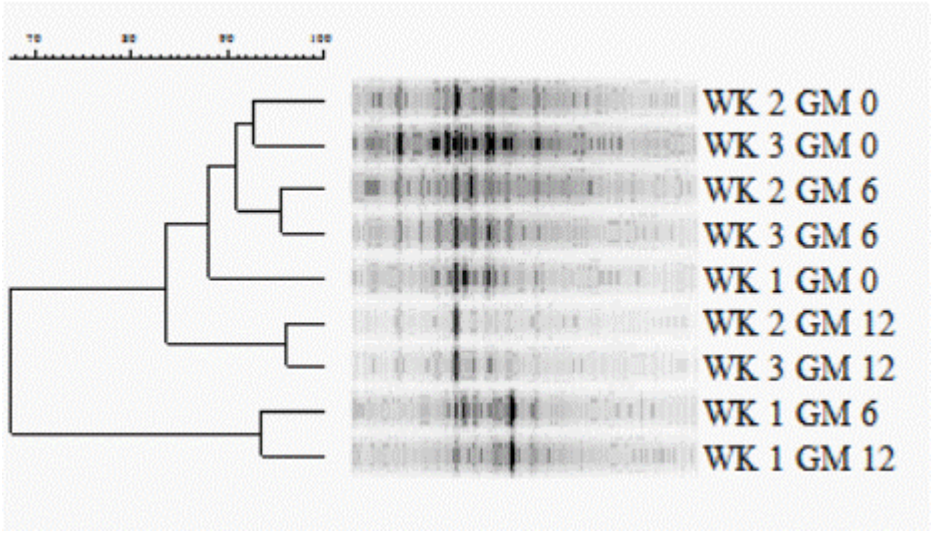


**FIGURE 6-3. Denaturing gradient gel electrophoresis of bacterial 16S rDNA amplicons band patterns from adult cockerels consuming 0, 6, and 12% GM diets over a three week period of time. The bar above figure indicates percentage similarity coefficients.**

of one or a limited number of health-promoting bacterial in the intestinal tract, thus improving the host's intestinal balance." Some of the most commonly studied prebiotics in human and terrestrial animals include fructo-oligosaccharide products (FOS, oligofructose, inulin), galacto-oligosaccharides (Patterson and Burkholder, 2003), and manno-oligosaccharides (MOS) which do not selectively enrich for beneficial bacterial populations but rather remove pathogens from the intestinal tract and stimulate the immune system (Spring et al., 2000).

Guar gum is a soluble polysaccharide that is resistant to enzymatic digestion in the small intestine but is easily degradable by the resident microbes in the large bowel of humans (Macfarlane and Macfarlane, 1993). Guar gum modifies the GI microbial community, to enhance non-specific immune responses (Bailey et al., 1991), and increase fermentation products (Smiricky-Tjardes et al., 2003) as well as improve mineral uptake and performance indices such as feed conversion and protein efficiency ratios (Bongers and van de Heuvel, 2003).

In the current experiment, once acclimated to their respective diets, chickens consuming the 12% GM diet exhibited a considerable change in the composition of cecal bacterial flora relative to birds consuming 0 and 6% GM diets. While inconsistent patterns were noted between treatment groups during the first week of the trial (Figure 6-1), bacterial populations increased in SC between wk 2 and 3. Hume et al. (2003) reported a similar observation in adult laying hens whose diet was abruptly changed to one containing high levels of alfalfa.

Additionally, an apparent decrease in complexity of the intestinal microbiota was observed as time increased in birds consuming the 12% GM diet (Figure 6-1). Generally, a more complex microbiota is considered to be indicative of good GI health (McNab, 1973) although birds consuming the 12% GM diet in this trial appeared to be in good health with otherwise normal-appearing excreta. Slavin and Greenberg (2003) reported a similar decrease in microbial population complexity with specific increases in Bifidobacterium present in the feces of human subjects. One hypothesis is that the binding capacity of guar gum to some types of bacteria (e.g. *Salmonella, Shigella*, and *Enterobacteriacae*) caused a portion of this decrease in cecal ecology. However, the degree to which this change is visualized on the molecular fingerprinting software is quite dramatic and likely to be at least partially explained by other unknown factors.

Recently, there has been a renewed interest in GI microbial ecology in which culture-based microbial techniques are increasingly being supplemented with molecular techniques (Tannock, 1999). Theoretically, methods based on the 16S rDNA gene can be used to identify all bacterial genus or species within gastrointestinal microbial communities whereas cultivation approaches are biased due to the inability of some bacterial to grow on selective media, thereby excluding them from further analysis (Amann et al., 1995). The limitation of PCR and cloning to provide accurate quantitative measurements of the actual composition of a microbial community are well documented (Farrelly et al., 1995; Suzuki and Giovannoni, 1996). Primary among these limitations is the possibility of co-migration of amplicons of varied G-C content and primary DNA sequence in the denaturing gradient (Wintzingerode et al., 1997),

potentially resulting in an inaccurate indication of genotypic diversity and/or abundance. Despite the small numbers of dark bands detected, differences in amplicon profiles allowed visualization of changes in predominant cecal microbiota as birds grew accustomed to their respective diets.

Visualization of modifications in digestive community profiles may have useful diagnostic implications although real differences in poultry digestive populations can only be determined through identification of microbial components. This can be accomplished by amplicon cloning and sequence comparisons with other 16S gene sequences (Burr et al., 2008; Zhu et al., 2002). This type of analysis was beyond the scope of the current experiment but should be strongly considered in future trials which evaluate changes in intestinal ecology.

# CHAPTER VII

# SUMMARY

Understanding the effects of soluble and insoluble non-starch polysaccharides (NSP) on digestive physiology is fundamental to their incorporation into commercial poultry diets. Structures and physicochemical characteristics of NSP, such as viscosity and water-holding capacity differ widely among feed ingredients and affect digestive processes by different means (Wenk, 2001). Several authors suggest that gut adaptation modifies the gastrointestinal effects of poorly digestible carbohydrates (Rao et al., 1994; Weaver et al., 1996). Rats fed different types of indigestible polysaccharides required 3-12 wk to stabilize cecal bacterial mass and metabolic activity before stable digestibility measurements of NSP and cecal SCFA concentrations could be acquired (Brunsgaard et al., 1995). In humans, a similar response was reported in which an 8-d dosing schedule of lactulose was better digested than a single dose (Florent et al., 1985) and reduced diarrhea induced by a single large dose (Flourie et al., 1993).

Certain soluble fibers also are prebiotic in nature and constitute an important source for bacterial fermentation, from which products such as hydrogen, methane, carbon dioxide, and short chain fatty acids are produced. These fermentation products influence glucose and fat metabolism, reduce postprandial glycemia, and reduce serum concentrations of free fatty acids and cholesterol. As a result of fiber consumption, proliferation of mucosal epithelial cells in both the ceca and colon increase and intraluminal pH decreases significantly (Macfarlane and Cummings, 1991).

Guar gum is the galactomannan of the endosperm of guar beans (*Cyamopsis tetragonoloba*). Galactomannans are present in a number of sources in nature including the locust bean, alfalfa seeds, soybeans, sugar beets, and coffee beans (Slavin and Greenberg, 2003). In the food industry, guar gum is used as a thickening and stabilizing agent in a wide variety of foods, usually in amounts less than 1% of food weight. Although guar gum has positive physiologic benefits, its high viscosity makes it difficult to incorporate into food products at high levels. In a preliminary experiment, adult cockerels consuming increasing levels of guar meal (GM) showed an increase in its metabolizable energy content occurring about 20 days into the trial period when their diet contained between 10 and 30% dietary GM. The experiments presented in this paper evaluated the physiological changes that occurred in cockerels consuming GM over a 3-week acclimation period which might explain this observation.

In experiment 1, birds were administered diets containing 0, 6, 12, or 24% GM for a 3-wk period. Following the 3-wk acclimation period, birds were killed and differences in digestive system organ weight and size were determined. Body weight, feed consumption and intestinal histological parameters were also measured. Significant changes in small intestine, liver, pancreas and overall body weight in addition to cecal length, villus height and mucosal lesion score were observed in birds consuming the 24% GM diet relative to the control group. This indicates that at sufficiently high dietary levels (24%), the NSP content of GM increased the physical size of the GI tract and associated digestive organs as has been observed in animals consuming bulky,

fibrous diets. However, as indicated by the decrease in body weight of the 24% GM group, net digestibility is likely decreasing.

In experiment 2, a partial cross-over experiment was conducted in which birds were given a 3-wk acclimation period to diets containing either 0 or 24% GM. This was followed by a series of nitrogen-corrected true metabolizable energy (TMEn) bioassays to determine whether the acclimation period affected energy utilization of GM. In each of the cross-over phases, a significant decrease in the TMEn of GM was observed as a result of consuming the 24% GM diet. Furthermore, significant increases in excreta energy were noted when birds were subjected to the 24% GM diet. This indicated that the decrease in energy utilization resulted from a gross inability to absorb these nutrients from the lumen of the GI tract into the body. Thus, exposure to high levels of GM damaged the GI tract to an extent that the bird was unable to recuperate, much less acclimate to its new diet. This damage is most likely due to the rheological properties of GG which imbibes water with very high affinity and affects osmolarity within the intestinal lumen. Due to the weight loss observed in birds consuming 24% GM over the 3- wk period, it is unknown whether a truly "GM-preconditioned" state is actually possible for such elevated dietary concentrations.

In experiment 3, differences in TMEn values of GM were evaluated in birds consuming 0, 6, and 12% GM diets with respect to the manner in which endogenous energy losses (EEL) were calculated and incorporated into the TMEn equation. In comparing "self-corrected" EEL values versus the more traditional "group-corrected" EEL method, significant differences in the TMEn value of GM were observed. When

calculated conventionally (i.e. using the group-corrected EEL term), pre-conditioning diet did not result in differences in TMEn value. However, when self-corrected EEL values were employed, a significant increase in TMEn content was observed in the 12% GM group relative to control and 6% GM groups. Furthermore, significant changes in the EEL of birds consuming different levels of GM indicate that a self-correction should be employed when determining TMEn of ingredients rich in soluble fibers such as GM.

In experiment 4, changes in the cecal microbial population of cockerels consuming 0, 6, and 12% GM over a 3-wk period of time were determined using a denaturing gradient gel electrophoretic procedure on amplified bacterial 16S rDNA. After an initial adjustment period of just over 1 week in which the microbial populations seemed to be in a state of flux, discernable patterns emerged among the treatment groups. By wk 2, birds consuming 12% dietary GM showed differences in cecal bacterial profile when compared to the control and 6% GM groups, which were more similar in microbial community.

Changes in TMEn value of GM with few associated phenotypic changes in GI morphology indicates that some factor which assists the digestive and absorptive processes is triggered in birds consuming relatively high levels of GM (12%) but not those consuming lesser amounts (6% GM). The change in cecal microbiota which resulted from consuming the 12% GM diet could explain this observation. Although cecal microbial populations are considered to be relatively stable in adult animals, they appear to require a 1-2 wk adjustment period when administering “functional” foods such as guar gum.

It can be argued that the effects seen in these trials are due to factors contained within GM other than NSP (i.e. saponin). While this can not be excluded as a possibility since no purified NSP source (GG) was evaluated on a side-by-side basis, it is the opinion of this author that the likelihood of such dramatic physical changes being caused by something other than GG is low. While the effects of saponin consumption indicate a number of significant changes within the GI tract of animals (Francis et al., 2002), a substantial number of reports exist which show similar trends as were noted in the present report in several animal species administered both purified and mixed NSP sources in response to dose and time (Choct, 1997; Annison and Choct, 1991).

Previously reported energy values of ~2000 Kcal/Kg (Ambegaokar et al., 1969) seem to underestimate the actual TMEn content of GM based on the fact that birds consuming 6 and 12% GM did not lose weight and in some cases gained weight during the 3-wk preconditioning period. Furthermore, our assays consistently showed GM TMEn values upwards of 2800 Kcal/Kg. Regrettably, the energy values obtained in this series of studies are quite high relative to that of other vegetable-based protein sources, and may be criticized as being artificially high. Unfortunately a side-by-side comparison of well-known ingredients (i.e., soybean meal or corn gluten meal) was not conducted in order to standardize our calculation of the TME content of GM. The use of this type of reference standard in future evaluations is probably worthwhile.

One strategy which was not examined thoroughly but in hind sight, should have been was the use of a “step-up” program in which birds undergo a more gradual introduction to high levels of GM by steadily increasing the amount of dietary GM over

the course of the preconditioning period. It was in this manner, that the original hypothesis of the impact of preconditioning period came about, and is more consistent with what would occur in the natural environment than the immediate dosing approach that was employed in each of the experiments reported here. It is likely that the preconditioning period of 3 weeks was not sufficient to allow an adequate, favorable physiological response to the high levels of GM. Further, it is almost certain that both the "slug-dosing" schedule (as opposed to a step up schedule) combined with the brief preconditioning period (relative to observations in wild galliforms) resulted in less than favorable results with respect to determining the birds' ability to truly acclimate to diets containing high levels of GM. Adding to the difficulty of these analyses is the presence of saponin which is at the very least a confounding factor in determining the physiological effects of consuming GM.

These experiments demonstrate that consuming moderate to high levels of GM affects the digestive physiology of adult cockerels influencing both GI morphology and micro-ecology. However, this change in physiology did not result in a predictable, overall increase in GM utilization. Although the consumption of up to 12% dietary GM did not result in morphological changes to the GI tract, notable differences in cecal microbial communities coupled with consistent increases in the TMEn of GM were observed relative to birds consuming 6% GM. This suggests that a physiological threshold may exist in which GM utilization actually increases once a certain "bioactive level" is attained. The bioactivity of GM is likely a combination of NSP and saponin activity which seem to occur at differing levels of inclusion, creating a delicate

balancing act between beneficial and detrimental effects, and it should be noted that 24% GM seemed to exceed the upper limit of "bioactive tolerance," resulting in decreased vitality evidenced as decreased GI absorptive capacity and body weight loss (experiments 1 and 2). Further, it seems clear that the effects of GM on GI physiology are a function of acclimation time, adding another dynamic to the already complicated formula, in that birds may respond differently to various fiber sources depending upon acclimation schedule and timeframe. Non-starch polysaccharide content of ingredients must thus be monitored and managed to ensure that predictable growth/production performance is attained, particularly in animals which have a relatively short production period.

# REFERENCES

Adeola, O., D. Ragland, and D. King. 1997. Feeding and excreta collection techniques in metabolizable energy assays for ducks. Poult. Sci. 76:728-732.

Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143–169.

Ambegaokar, S. D., J. K. Kamath, and V. P. Shinde. 1969. Nutritional studies in protein of 'gawar' (*Cyamopsis tetragonoloba*). J. Nutr. Diet. 6:323-328.

Anderson, J. O., and R. E. Warnick. 1964. Value of enzyme supplements in rations containing certain legume seed meals or gums. Poult. Sci. 43:1091-1097.

Annison, E. F., K. J. Hill, and R. C. Kenworthy. 1968. Volatile fatty acids in the digestive tract of the fowl. Br. J. Nutr. 22:207-216.

Annison, G., and M. Choct. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effect. World's Poult. Sci. J. 47:232–242.

Bailey, J., L. Blankenship, and N. Cox. 1991. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poult. Sci. 70:2433–2438.

Bailey, R.W. 1973. Structural carbohydrates. Pages 157-211 in: Chemistry and Biochemistry of Herbage. Vol. 1. G. W. Butler, G. W., and R. W. Bailey, eds. Academic Press, London.

Bakshi, Y. K. 1966. Studies on toxicity and processing of guar meal. PhD Diss. Texas A&M Univ., College Station, TX.

Bakshi, Y. K., C. R. Creger, and J. R. Couch. 1964. Studies on guar meal. Poult. Sci. 43:1302. (Abstr.)

Bakshi, Y. K., J. M. Prescott, C. R. Creger, and J. R. Couch. 1965. Evaluation of guar meal. Poult. Sci. 44:1350. (Abstr.)

Barnes, E. M. 1979. The intestinal microflora of poultry and game birds during life and after storage. J. Appl. Bacteriol. 45:407– 419.

Barnes, E. M., G. C. Smith, D. A. Barnum, and E. G. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311–326.

Beattie, J., and D. H. Shrimpton. 1958. Surgical and chemical techniques for in vivo studies of the intestinal microflora of domestic fowls. Exp. Physiol. 43:399.

Bedbury, H. P., and G. E. Duke. 1983. Cecal microflora of turkeys fed low or high fiber diets: enumeration, identification and determination of cellulolytic activity. Poult. Sci. 62:675-682.

Bedford, M. R., H. L. Classen, and G. L. Campbell. 1991. The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. Poult. Sci. 70:1571-1577.

Bengmark, S. 1998. Immunonutrition: role of biosurfactants, fiber, and probiotic bacteria. Nutrition. 14:585-594.

Blackburn, N. A., and I. T. Johnson. 1981. The effect of guar gum on the viscosity of the gastrointestinal contents and on glucose uptake from the perfused jejunum in the rat. Br. J. Nutr. 46:239-246.

Bongers, A., and E. G. H. M. van den Heuvel. 2003. Prebiotics and the bioavailability of minerals and trace elements. Food Rev. Int. 19:397–422.

Borchers, R., and C. W. Ackerson. 1950. The nutritive value of legume seeds: effect of autoclaving and the trypsin inhibitor test for 17 species. J. Nutr. 41: 339.

Borin, K., J. E. Lindberg, and R. B. Ogle. 2006. Digestibility and digestive organ development in indigenous and improved chickens and ducks fed diets with increasing inclusion levels of cassava leaf meal. J. Anim. Physiol. Anim. Nutr. 90:230-237.

Brouns, F., B. Kettlitz, and E. Arrigoni. 2002. Resistant starch and "the butyrate revolution". Trends Food Sci. Technol. 13:251-261.

Brunsgaard, G., K. E. Bach-Knudsen, and B. O. Eggum. 1995. The influence of the period of adaptation on the digestibility of diets containing different types of indigestible polysaccharides in rats. Br. J. Nutr. 74:833–848.

Burr, G., M. Hume, S. Ricke, D. Nisbet, and D. Gatlin III. 2008. A preliminary in vitro assessment of GroBiotic®-A, brewer's yeast and fructooligosaccharide as prebiotics for the red drum (*Sciaenops ocellatus*). J. Environ. Sci. Health. 43:253-260.

Carre, B., L. Derouet, and B. Leclercq. 1990. The digestibility of cell-wall polysaccharides from wheat (bran or whole grain), soybean meal, and white lupin meal in cockerels, muscovy ducks, and rats. Poult. Sci. 69:623–633.

Chiu, C. J., A. H. McArdle, R. Brown, H. J. Scott, and F. N. Gurd. 1970. Intestinal mucosal lesion in low-flow states. Arch. Surgery 101:478-483.

Choct, M. 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. Feed Mill. Int. 13–27.

Choct, M., and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. Br. Poult. Sci. 33:821-834.

Coates, M. E., J. E. Ford, and G. F. Harrison. 1968. Intestinal synthesis of vitamins of the B complex in chicks. Br. J. Nutr. 22:493.

Collip, J. B., 1922. The activation of the glandular stomach of the fowl. Am. J. Physiol. 59:435-438.

Conner, S. R. 2002. Characterization of guar meal for use in poultry rations. PhD Diss. Texas A&M Univ., College Station, TX.

Couch, J. R., Y. K. Bakshi, T. M. Ferguson, E. B. Smith, and C. R. Creger. 1967. The effect of processing on the nutritional value of guar meal for broiler chicks. Br. Poult. Sci. 8:243-250.

Cummings, J. H., and G.T. Macfarlane. 1997. Role of intestinal bacteria in nutrient metabolism. J. Parenter. Enteral Nutr. 21:357–365.

Dario-Frias, A. C., and V. C. Sgarbieri. 1998. Guar gum effects on food intake, blood serum lipids and glucose levels of wistar rats. Plant Foods Hum. Nutr. 53:15-28.

Don, R. H., P. T. Cox, B. J.Wainwright, K. Baker, and J. S.Mattick. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19:4008.

Dowling, R. H., E. O. Reicken, J. W. Laws, and C. C. Booth. 1967. The intestinal response to high bulk feeding in the rat. Clin. Sci. 32:1-9.

Duke, G. E., 1986. Alimentary canal: secretion and digestion, special digestive functions, and absorption. Pages 289-300 in: Avian Physiology. 4th ed. P. D. Sturkie, ed. Springer-Verlag, New York.

Duke, G.E., E. Eccleston, S. Kirkwood, C. F. Louis, and H. P. Bedbury. 1984. Cellulose digestion by domestic turkeys fed low or high fiber diets. J. Nutr. 114:95-102.

Eastwood, M. A. 1992. The physiological effect of dietary fiber: an update. Annu. Rev. Nutr. 12:19-35.

Eastwood, M. A., W. G. Brydon, and D. M. W. Anderson. 1986. The effect of the polysaccharide composition and structure of dietary fibers on cecal fermentation and fecal excretion. Am. J. Gen. Nutr. 44:5l-55.

Edwards, C. A. 1993. Interactions between nutrition and intestinal microflora. Proc. Nutr. Soc. 52:375-382.

Edwards, C. A., I. T. Johnson, and N. W. Read. 1988. Do viscous polysaccharides reduce absorption by inhibiting diffusion or convection? Eur. J. Clin. Nutr. 42:307-3l 2.

Farner, D. S. 1960. Digestion and the digestive system. Pages 411-467 in: Biology and Comparative Physiology of Birds. Vol. l. A. J. Marshall, ed. Academic Press, New York.

Farrell, D. J. 1978. Rapid determination of metabolizable energy of foods using cockerels. Br. Poult. Sci. 19:303–308.

Farrelly, V., F. A. Rainey, and E. Stackebrandt. 1995. Effect of genome size and *rrn* gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl. Environ. Microbiol. 61:2798–2801.

Favier, M. L., P. E. Bost, C. Demigne, and C. Remesy. 1998. The cholesterol-lowering effect of guar gum in rats is not accompanied by an interruption of bile acid cycling. Lipids 33:765-771.

Favier, M. L., P. E. Bost, C. Guittard, C. Demigne, and C. Remesy. 1997. Reciprocal influence of fermentations and bile acid excretion on cholesterol-lowering effect of fermentable carbohydrate. J. Nutr. Biochem. 8:127-132.

Fenna, L., and D. A. Boag. 1974. Adaptive significance of the caeca in japanese quail and spruce grouse (Galliformes). Can. J. Zool. 52:1577-1584.

Fernandez, F., M. Hinton, and B. van Gils. 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonization. Avian Pathol. 31:49-58.

Fischer, S. C., and L. S. Lerman. 1983. DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. Proc.Natl. Acad. Sci. 80:1579–1583.

Florent, C., B. Flourie, A. Leblond, M. Rautureau, J. J. Bernier, and J. C. Rambaud. 1985. Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). J. Clin. Invest. 75:608-613.

Flourie, B., F. Briet, C. Florent, P. Pellier, M. Maurel, and J. C. Rambaud. 1993. Can diarrhea induced by lactulose be reduced by prolonged ingestion of lactulose?. Am. J. Clin. Nutr. 58:369-375.

Francis, G., Z. Kerem, H. P. Makkar, and K. Becker. 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88:587–605.

Francis, G., H. P. Makkar, and K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 19:197–227.

Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Gasaway, W. C. 1976. Volatile fatty acids and metabolizable energy derived from cecal fermentation in the willow ptarmigan. Comp. Biochem. Physiol. 53:115-121.

Gee, J. M., and I. T. Johnson. 1988. Interactions between haemolytic saponins, bile salts and small intestinal mucosa in the rat. J. Nutr. 118:1391–1397.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125:1401-1412.

Goldstein, D. L. 1989. Absorption by the cecum of wild birds: is there interspecific variation. J. Exp. Zool. (Suppl. 3):103–110.

Gonzalez-Esquerra, R., and S. Leeson. 2000. Studies on the metabolizable energy content of ground full-fat flaxseed fed in mash, pellet, and crumbled diets assayed with birds of different ages. Poult. Sci. 79:1603–1607.

Guillaume, J., and Summers J. D. 1970. Maintenance energy requirement of rooster and influence of plane of nutrition on metabolisable energy. Can. J. Anim. Sci., 50:363–369.

Gutierrez, O., C. Zhang, D. J. Caldwell, J. B. Carey, A. L. Cartwright, and C.A. Bailey. 2008. Guar meal diets as an alternative approach to inducing molt and improving *Salmonella enteritidis* resistance in late-phase laying hens. Poult. Sci. 87:536-540.

Gutierrez, O., C. Zhang, A. L. Cartwright, J. B. Carey, and C. A. Bailey. 2007. Use of guar by product in high production laying hen diets. Poult. Sci. 86:1115–1120.

Halnan, E. T. 1949. Architecture of the avian gut and tolerance of crude fibre. Br.J. Nutr. 3:245.

Hanssen, I. 1979. Micro-morphological studies on the small intestine and ceca in wild and captive willow grouse (*Lagopus lagopus*). Acta Vet. Scand. 20:351-364.

Harris, L. E. 1966. Biological energy interrelationships and glossary of energy terms, N.A.S. - N.R.C. Publication 1411, Washington DC.

Hassan, S. M., J. A. Byrd, A. M. Berhow, C. A. Bailey, and A. L. Cartwright. 2007. Saponin rich extracts from quillaja, yucca, soybean, and guar differ in antimicrobial and hemolytic activities. Poult. Sci. 86 (Suppl 1):121. (Abstr.)

Herd, R. M., and T. J. Dawson. 1984. Fiber digestion in the emu (*Dramaius novaehollandiae*) a large ratite bird with a simple gut and high rates of passage. Physiol. Zool. 57:70–84.

Hill, F. W., and D. L. Anderson, 1958. Comparison of metabolizable energy and productive determinations with growing chicks. J. Nutr. 64:587–603.

Hume, M. E., L. F. Kubena, T. S. Edrington, C. J. Donskey, R. W. Moore, S. C. Ricke, and D. J. Nisbet. 2003. Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult. Sci. 82:1100-1107.

Iji, P. A. 1999. The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. World's Poult. Sci. J. 55:375-387.

Ikeda, K., and K. Kusano. 1983. In vitro inhibition of digestive enzymes by indigestible polysaccharides. Cereal Chem. 60:260–263.

Ikegami, S., F. Tsuchihashi, H. Harada, N. Tsuchihashi, E. Nishide, and S. Innami. 1990. Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. J. Nutr. 120:353-360.

Inman, D. L. 1973. Cellulose digestion in ruffed grouse, chukar partridge, and bobwhite quail. J. Wildl. Manage. 37:114-121.

Ishihara, N., D. C. Chu, S. Akachi, and L. R. Juneja. 2000. Preventive effect of partially hydrolyzed guar gum on infection of *Salmonella enteritidis* in young and laying hens. Poult. Sci. 79:689-697.

Jamroz, D., K. Jakobsen, J. Orda, J. Skorupinska, and A. Wiliczkiewicz. 2001. Development of gastrointestinal tract and digestibility of dietary fibre and amino acids in young chickens, ducks and geese fed diets with amounts of barley. Comp. Bioch. Physiol. 130:643-652.

Jamroz, D., A. Wiliczkiewicz and J. Skorupinska. 1998. Fermentation and apparent digestion of the structural carbohydrates in chicks, ducks, and geese fed triticale mixtures supplemented with enzymes. J. Anim. Physiol. Anim. Nutr. 79:1–17.

Johansson, K. R., W. B. Sarles, and S. K. Shapiro. 1948. The intestinal microflora of hens as influenced by various carbohydrates in a biotin-deficient ration. J. Bacteriol. 56:619-633.

Johnson, I. T., and J. M. Gee. 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. Gut 22:398-403.

Jorgensen, H., X.Q. Zhao, K.E. Knudsen, and B.O. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility, and energy metabolism in broiler chickens. Br. J. Nutr. 75:379–395.

Kakani, R., O. Gutierrez, A. Haq, and C.A. Bailey. 2007. Extraction of saponins from guar meal. Poult. Sci. 86(Suppl. 1):314. (Abstr.)

Kirgchgessner, M., K. Eder, H. L. Müller, and D. Jamroz. 1999. Zur energetichen bewertung von nichtstärke polisacchariden beim geflügel. J. Anim. Physiol. Anim. Nutr. 81:8151–8155.

Krogdahl, A., T. B. Lea, J. L. Olli. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 107:215– 219.

Langhout, D. J., and J. B. Schutte. 1996. Nutritional implications of pectins in chickens in relation to estrification and origin of pectins. Poult. Sci. 75:1236–1242.

Larbier, M., and B. Leclercq. 1992. Nutrition et alimentation des volailles. INRA Paris. English Edition: Nutrition and Feeding of Poultry. Translated and Edited by J. Wiseman, Nottingham University Press, Nottingham, United Kingdom.

Lee, J. T., C. A. Bailey, and A. L. Cartwright. 2003. Guar meal germ and hull fractions differently affect growth performance and intestinal viscosity of broiler chickens. Poult. Sci. 82:1589-1595.

Lee, J. T., S. Connor-Appleton, C.A. Bailey, and A.L. Cartwright. 2005. Effects of guar meal by-product with and without beta-mannanase Hemicell® on broiler performance. Poult. Sci. 84:1261–1267.

Levine, G. N., J. J. Derin, E. Steiger, and R. Zinno. 1974. Role of oral intake and maintainence of gut mass and disaccharide activity. Gastroenterology 67:975-982.

Livesey, G. 1990. Energy values of unavailable carbohydrates and diets: an inquiry and analysis. Am. J. Clin . Nutr. 51:617-637.

Lodhi, G. N., R. Renner, and D. R. Clandinin. 1969. Studies on the metabolizable energy of rapeseed meal for growing chickens and laying hens. Poult. Sci. 48:964–970.

Macfarlane, G. T., and J. H. Cummings. 1991. The colonic flora, fermentation and large bowel digestive function. Pages 51-92 in: The Large Intestine: Physiology, Pathophysiology and Disease. S. F. Phillips, J. H. Pemberton, and R. G. Shorter, eds. Raven Press, New York.

Macfarlane, G. T., and S. Macfarlane. 1993. Factors affecting fermentation reactions in the large bowel. Proc. Nutr. Soc. 52:367-373.

McBee, R. H. 1977. Fermentation in the hindgut. Pages 185-250 in: Microbial Ecology of the Gut. R. T. J. Clark, and T. Bauchop, eds. Academic Press, New York.

McBee, R. H., and G. C. West. 1969. Caecal fermentation in the willow ptarmigan. Condor 71:54.

McCullough, J. S., B. Ratcliffe, N. Mandir, K. E. Carr, and R. A. Goodlad. 1998. Dietary fibre and intestinal microflora: effects on intestinal morphometry and crypt branching. Gut 42:799–806.

McHan, F., and E. B. Shotts. 1993. Effect of short-chain fatty acids on the growth of *Salmonella typhimurium* in an in vitro system. Avian. Dis. 37:396–398.

McNab, J. M. 1973. The avian caeca: a review. World's Poult. Sci. J. 29:251-263.

McNab, J. M. 1989. Measuring availability of amino acids from digestibility experiments. Proc. 7th European Symp. Poultry Nutr., World's Poult. Sci. Assoc., Girona, Spain.

McNab, J. M. 1999. Energy supply to poultry. J. Exp. Zool. 283:377-386.

McNab, J. M., and J. C. Blair. 1988. Modified assay for true and apparent metabolizable energy based on tube feeding. Br. Poult. Sci. 29:697–707.

McNab, J. M., and C. Fisher. 1982. The choice between apparent and true metabolizable energy systems – recent evidence. 3rd European Symp. Poultry Nutr., World's Poult. Sci. Assoc., Edinburgh, Scotland.

Mead, G. C. 1974. Anaerobic utilization of uric acid by some group D streptococci. J. Gen. Microbiol. 82:421-423.

Mead, G. C. 1989. Microbes of the avian cecum: types present and substrates utilized. J. Exp. Zool. (Suppl. 3):48–54.

Mead, G. C. 1997. Bacteria in gastrointestinal tract of birds. Pages 216-242 in: Gastrointestinal Microbiology, Gastrointestinal Microbes and Host Interactions. R. Mackie, B. A. White, and R. E. Isaacson, eds. Chapman and Hall, New York.

Mead, G. C., and B. W. Adams. 1975. Some observation on the caecal microflora of the chick during the first two weeks of life. Br. Poult. Sci. 16:169-176.

Miller, M. R. 1976. Cecal fermentation in mallards in relation to diet. Condor 78:107-111.

Miller, M. R., and K. J. Reinecke. 1986. Proper expression of metabolizable energy in avian energetics. Condor 86:396-400.

Mohamed, K., M. Larbier, and B. Leclercq. 1986. A comparative study of the digestibility of soybean and cottonseed amino acids in domestic chicks and muscovy ducklings. Anal. Zootech. 35:79-86.

Moriceau, S., C. Besson, M. A. Levrat, C. Moundras, C. Remesy, C. Morand, and C. Demigne. 2000. Cholesterol-lowering effects of guar gum: changes in bile acid pools and intestinal reabsorption. Lipids 35:437-444.

Morton, E. S. 1978. Avian arboreal folivores: why not? Pages 123-130 in: Ecology of Arboreal Folivores. G. G. Montgomery, ed. The Smithsonian Institution, Washington DC.

Moss, R. 1977. The digestion of heather by red grouse during the spring. Condor 79:471-477.

Moss, R. and I. B. Trenholm. 1987. Food intake digestibility and gut size in red grouse. Br. Poult. Sci. 28:81-89.

Murray, A. E., J. T. Hollibaugh, and C. Orrego. 1996. Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. Appl. Environ. Microbiol. 62:2676–2680.

Muyzer, G., E. C. De Waal, and A. G. Litterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase-chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695–700.

Nagpal, M. L., O. P. Agrawal, and I. S. Bhatia. 1971. Chemical and biological examination of guar meal (*Cyamopsis tetragonoloba*). Ind. J. Anim. Sci. 41:283-293.

Nagra, S. S., B. K. Shingari, and J. S. Ichhponani. 1985. Feeding of guar (*Cyamopsis tetragonoloba*) meal to poultry. 1. Growth of commercial broiler chicks. Ind. J. Poult. Sci. 20:188-193.

Netherwood, T., H. J. Gilbert, D. S. Parker, and A. G. O'Donnell. 1999. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Appl. Environ. Microbiol. 65:5134–5138.

Noack, J., B. Kleesen, J. Proll, G. Dongowski, and M. Blaut. 1998. Dietary guar gum and pectin stimulate intestinal microbial polyamine synthesis in rats. Nutrition 128:1385– 1391.

Okubo, T., N. Ishihara, H. Takahashi, T. Fujisawa, M. J. Kim, T. Yamamoto, and T. Mitsuoka. 1994. Effects of partially hydrolyzed guar gum intake on human intestinal microflora and its metabolism. Biosci. Biotechnol. Biochem. 58:1364-1369.

Oleszek, W., J. Nowacka, J. M. Gee, G. Wortley, and I. T. Johnson. 1994. Effects of some purified alfalfa (*Medicago sativa* ) saponins on transmural potential difference in mammalian small intestine. J. Sci. Food Agri. 65:35–39.

Patel, M. B., M. S. Jami, and J. McGinnis. 1980. Effect of gamma irradiation, penicillin, and/or pectic enzyme on chick growth depression and fecal stickiness caused by rye, citrus pectin, and guar gum. Poult. Sci. 59:2105-2110.

Patel, M. B., and J. McGinnis. 1985. The effect of autoclaving and enzyme supplementation of guar meal on the performance of chicks and laying hens. Poult. Sci. 64:1148-1156.

Patterson, J. A., and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Potter, S. M., R. Jimenez-Flores, J. Pollack, T. A. Lone, and M. D. Berber-Jimenez. 1993. Protein saponin interaction and its influence on blood lipids. J. Agric. Food Chem. 41:1287–1291.

Prop, S., and T. Vulnik. 1992. Digestion by barnacle geese in the annual cycle: the interplay between retention time and food quality. Funct. Ecol. 6:180–189.

Pryde, S. E., A. J. Richardson, and C. S. Steward. 1999. Molecular analysis of the microbial diversity present in the colonic wall, colonic lumen and cecal lumen of a pig. Appl. Environ. Microbiol. 65:5372-5377.

Pulliainen, E. 1976. Small intestine and caeca lengths in the Willow Grouse (*Lagopus lagopus*) in Finnish Lapland. Ann. Zool. Fenn.13:195-199.

Radeff, T. 1928. Uber die Rohfaserverdauung beim Huhn und die Hierbei die linddärm Zukommende Redeutung. Biochem. Zool. 193:192.

Rahner, R. 1901. Bakteriologische mitteilungen ueber die darmbacterien der huehner. Zentbl. Bakteriol. Parasitenkd. 193:80-95.

Rainbird, A. L., and A. G. Low. 1986. Effect of guar gum on gastric emptying in growing pigs. Br. J. Nutr. 55:87-98.

Rao, P. V., and D. R. Clandinin. 1970. Effect of method of determination on metabolizable energy value of rapeseed meal. Poult. Sci. 49:1069-1074.

Rao, P. V., N. Shiwnarain, M. Koo, and D. J. A. Jenkins. 1994. Effect of fiber-rich foods on the composition of intestinal microflora. Nutr. Res. 14:523-535.

Redig, P. 1989. The avian ceca: obligate combustion chambers or facultative afterburners? – The conditioning influence of diet. J. Exp. Zool. (Suppl. 3):66–69.

Roche, M. 1981. Feeding behaviour and digestive motility of birds. Reprod. Nutr. Devel. 21:781-789.

Roediger, W. E. W. 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa of man. Gut 21:793-798.

Rubio, L. A., and A. Brenes. 1988. Plasma mineral concentration in growing chicks fed diets containing raw and autoclaved faba beans (*Vicia faba*) and faba bean fractions. Nutr. Rep. Int. 38:609-617.

Russell, J. B. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Bacteriol. 73:363–370.

Salanitro, J. P., I. G. Blake, P. A. Muirhead, M. Maglio, and J. R. Goodman. 1978. Bacteria isolated from the duodenum of young chicks. Appl. Environ. Microbiol. 35:782-790.

Salanitro, J. P., I. G. Fairchilds, and Y. D. Zgornicki. 1974. Isolation, culture characteristics, and identification of anaerobic bacteria from the chicken cecum. Appl. Microbiol. 27:678-687.

Sathe, B. S., and S. Bose. 1962. Studies on the utilization of industrial and farm byproducts in growing poultry rations. Ind. J. Vet. Sci. 32:74-84.

Saxena, U. C., and K. Pradhan. 1974. Effect of high protein levels on the replacement value of guar meal (*Cyanopsis tetragonoloba*) in layer's ration [chickens]. Ind. J. Anim. Sci. 44:190-193.

Sell, J. L., L. G. Tenesaca, and G. L. Bales. 1979. Influence of dietary fat on energy utilization by laying hens. Poult. Sci. 58:900–905.

Sen, S., H. P. S. Makkar, S. Muetzel, and K. Becker. 1998. Effect of *Quillaja saponaria* saponins and *Yucca schidigera* plant extract on growth of *Escherichia coli*. Lett. Appl. Microbiol. 27:35–38.

Shah, N., R. R. Mahoney, and P. L. Pellett. 1986. Effect of guar gum, lignin and pectin on proteolytic enzyme levels in the gastrointestinal tract of the rat: a time-based study. J. Nutr. 116:786-794.

Shapiro, S. K., and W. B. Sarles. 1949. Microorganisms in the intestinal tract of normal chickens. J. Bacteriol. 58:531-544.

Shrimpton, D. H. 1963. Some volatile products of microbial metabolism in the caeca of the fowl. J. Appl. Baci. 26:1.

Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feeding stuffs. Poult. Sci. 55:303–308.

Sibbald, I. R. 1977. Effects of level of feed input, dilution of test material, and duration of excreta collection on true metabolizable energy values. Poult. Sci. 58:1325-1329.

Sibbald, I. R. 1979. Passage of feed through the adult rooster. Poult. Sci. 58:446-459.

Sibbald, I. R. 1980. The effects of dietary cellulose and sand on the combined metabolic plus endogenous energy and amino acid outputs of adult cockerel. Poult. Sci. 59:836–844.

Sibbald, I. R., J. Czarneck, S. J. Slinger, and G. G. Ashton. 1963. The prediction of metabolizable energy content of poultry feedingstuffs from a knowledge of their chemical composition. Poult. Sci. 57:557-558.

Sibbald I. R., and K. Price. 1977. True and apparent metabolizable energy values for poultry of Canadian wheats and oats measured by bioassay and predicted from physical and chemical data. Can. J. Anim. Sci. 57:365-374.

Sibbald, I. R., and K. Price. 1980. Variability in metabolic plus endogenous energy losses of adult cockerels and in the true metabolizable energy values and rates of passage of dehydrated alfalfa. Poult. Sci. 59:1275-1279.

Sibbald, I. R., and S. J. Slinger. 1962. The relationship between classical and corrected metabolizable energy values. Poult. Sci. 41:1007-1009.

Slavin, J. L., and N. A. Greenberg. 2003. Parially hydrolyzed guar gum: clinical nutrition uses. Nutrition 19:549-552.

Smiricky-Tjardes, M., M. C. Grieshop, E. Flickinger, L. Bauer, and G. Fahey Jr. 2003. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. J. Anim. Sci. 81:2535–2545.

Smith, C. H. M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52:203–221.

Southon, S., I. T. Johnson, J. M. Gee, and K. R. Price. 1988. The effect of *Gypsophylla* saponins in the diet on mineral status and plasma cholesterol concentration in the rat. Br. J. Nutr. 59:49–55.

Spring, P., C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella challenged broiler chicks. Poult. Sci. 79:205-211.

Stanogias, G., and G. R. Pearce. 1985. The digestion of fiber by pig. 2. Volatile fatty acid concentration in large intestine digesta. Br. J. Nutr. 53:513-536.

Stevens, E., and I. D. Hume. 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol. Rev. 78:393–427.

Sturkie, P. D. 1976. Avian Physiology. 3rd ed. Springer-Verlag, New York.

Suzuki, M. T., and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. 62:625-630.

Takahashi, H., S. Akachi, Y. Ueda, M. Kim, K. Hirano, and T. Yamamoto. 1995. Effect of liquid diets with or without partially hydrolyzed guar gum on intestinal microbial-flora and function of rats. Nutr. Res. 15:527-536.

Tannock, G. W. 1999. Analysis of the intestinal microflora: a renaissance. Antonie van Leeuwenhoek. 76:265-278.

Tannock, G. W. 2001. Molecular assessment of intestinal microflora. Am. J. Clin. Nutr. 73(Suppl):410S-414S.

Tenesaca, L. G., and J. L. Sell. 1981. Influence of an indigestible material on energy excretion by roosters and on true metabolizable energy of corn. Poult. Sci. 60:623-630.

Thakur, R. S., and K. Pradhan. 1975. Guar meal (*Cyamopsis tetragonoloba*) inclusion in broiler rations. effect on carcass yield and meat composition. Ind. J. Anim. Sci. 45:880-884.

Thornburn, C. C., and J. S.Willcox. 1965. The caeca of the domestic fowl and digestion of the crude fibre complex. 1. Digestibility trials with normal and caecectomised birds. Br. Poult. Sci. 6:23-31.

Topping, D. L. 1996. Short-chain fatty acids produced by intestinal bacteria. Asia Pac. J. Clin. Nutr. 5:15-19.

Tortuero, F., A. Brenas, and J. Riperez. 1975. The influence of intestinal (ceca) flora on serum and egg yolk cholesterol levels in laying hens. Poult. Sci. 54:1935–1938.

Tsukahara, P., and K. Ushida. 2000. Effects of animal or plant protein diets on cecal fermentation in guinea pigs (*Cavia porcellus*), rats (*Rattus norvegicus*) and chickens (*Gallus domesticus*). Comp. Bioch. Physiol. 127:139–146.

Vahouny, G. V. 1982. Dietary fiber, lipid metabolism and atherosclerosis. Fed. Proc. 41:2801-2806.

Van der Wielen, P. W., S. Biesterveld, L. J. A. Lipman, and F. van Knapen. 2001. Inhibition of a glucose-limited sequencing fed-batch culture of *Salmonella enterica* (Serovar Enteritidis) by volatile fatty representative of the ceca of broiler chickens. Appl. Environ. Microbiol. 67:1979–1982.

Van der Wielen, P. W., S. Biesterveld, S. Notermans, H. Hofstra, B. A. P. Urlings, and F. van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66:2536–2540.

Van Nevel, C. J., J. A. Decuypere, N. A. Dierick, and K. Molly. 2005. Incorporation of galactomannans in the diet of newly weaned piglets: effect on bacteriological and

some morphological characteristics of the small intestine. Arch. Anim. Nutr. 59:123-138.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Dairy Sci. 74:3583-3597.

Verma, S. V. S. 1977. The nutritive value of guar meal for poultry. PhD Thesis. University of Edinburgh, Scotland.

Verma, S. V. S., and J. M. McNab. 1982. Guar meal in diets for broiler chickens. Br. Poult. Sci. 23:95-105.

Verma, S. V. S., and J. M. McNab. 1984. Performance of hens fed guar meal containing diets with or without supplemental cholesterol. Ind. J. Poult. Sci. 19:245-250.

Vohra, P. 1966. Energy concepts for poultry nutrition. World's Poult. Sci. J. 22:6-24.

Vohra, P., and F. H. Kratzer. 1964. Growth inhibitory effect of certain polysaccharides for chickens. Poult. Sci. 43:1164-1170.

Wallace, R. J., L. Arthaud, and C. J. Newbold. 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60:1762-1767.

Weaver, G. A., C. T. Tangel, J. A. Krause, H. D. Alpern, P. L. Jenkins, M. M. Parfitt, and J. J. Stragand. 1996. Dietary guar gum alters colonic microbiotal fermentation in azoxymethane-treated rats. J. Nutr. 126:1979-1991.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Technol. 90:21-33.

Wintzingerode, F. V., U. B. Gobel, and E. Stackebrandt. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analyses. Microbiol. Rev. 21:213–229.

Yalcin, S., and A. G. Onol. 1994. True metabolizable energy values of some feedingstuffs. Br. Poult. Sci. 35:119–122.

Yamada, K., Y. Tokunaga, A. Ikeda, K. Ohkura, S. Mamiya, S. Kaku, M. Sugano, and H. Tachibana. 1999. Dietary effect of guar gum and its partially hydrolyzed product on the lipid metabolism and immune function of sprague-dawley rats. Biosci. Biotechnol. Biochem. 63:2163-2167.

Yamamoto, Y., I. Sogawa, A. Nishina, S. Saeki, N. Ichikawa, and S. Iibata. 2000. Improved hypolipidemic effects of xanthan gum - galactomannan mixtures in rats. Biosci. Biotechnol. Biochem. 64:2165-2171.

Yamauchi, K. 2002. Review of chicken intestinal villus histological alterations related with intestinal function. Jap. Poult. Sci. 39:229-242.

Yamauchi, K., K. Yamamoto, and Y. Isshiki. 1995. Morphological alterations of the intestinal villi and absorptive epithelial cells in each intestinal part in fasted chickens. Jap. Poult. Sci. 32:241-251.

Yu, B., C. C. Tsai, J. C. Hsu, and P. W. S. Chiou. 1998. Effect of different sources of dietary fibre on growth performance, intestinal morphology and caecal carbohydrases of domestic geese. Br. Poult. Sci. 39:560-567.

Zhu, X. Y., T. Zhong, Y. Pandya, and R. D. Joeger. 2002. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl. Environ. Microbiol. 68:124–137.

# APPENDIX I

## Nutrient matrix of guar meal used in these studies[1]

| | Guar meal |
|---|---|
| Moisture (%) | 7.00 |
| Crude protein (%) | 38.3 |
| ME (kcal/kg) | 2,033 |
| Calcium (%) | 0.16 |
| Available P (%) | 0.16 |
| Methionine (%) | 0.45 |
| Lysine (%) | 1.64 |
| Arginine (%) | 4.90 |
| Threonine (%) | 1.04 |
| Tryptophan (%) | 0.43 |

[1]Nutrient values represent a compilation of assayed guar by-products determined by Conner (2002), Office of the Texas State Chemist, Texas A&M Protein Chemistry Laboratory and the Degussa-Huls Corporation.

# VITA

**Omar Gutierrez**

Permanent Address:

15 Calgary Ct., Brownsville, Texas, 78526

Contact:

omagut@msn.com

Education:

1997 – 2001, Texas A&M University, B.S., Poultry Science

2004 – 2009, Texas A&M University, Ph.D., Poultry Science

Publications:

Gutierrez, O., N. Surbakti, A Haq, J.B. Carey, and C.A. Bailey. 2008. Effect of Intensive Multiphase Feeding on Broiler Performance and Nitrogen Excretion. J. Appl. Poult. Res. 17:463-470.

Gutierrez, O., C. Zhang, D.J. Caldwell, J.B. Carey, A.L. Cartwright, and C.A. Bailey. 2008. Guar Meal Diets as an Alternative Approach to Inducing Molt and Improving Salmonella Enteritidis Resistance in Late-Phase Laying Hens. Poult. Sci. 87:536-540.

Gutierrez, O., C. Zhang, A.L. Cartwright, J.B. Carey, and C.A. Bailey. 2007. Use of Guar By-Products in High Production Laying Hen Diets. Poult. Sci. 86:1115-1120.